2017

The root-associated microbiome and agricultural nitrogen use efficiency

Cassandra Wattenburger
Iowa State University

The root-associated microbiome and agricultural nitrogen use efficiency

by

Cassandra J. Wattenburger

A thesis submitted to the graduate faculty

in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

Major: Microbiology

Program of Study Committee:
Larry Halverson, Major Professor
Kirsten Hofmockel
Michael Castellano

The student author, whose presentation of the scholarship herein was approved by the program of study committee, is solely responsible for the content of this thesis. The Graduate College will ensure this thesis is globally accessible and will not permit alterations after a degree is conferred.

Iowa State University

Ames, Iowa

2017

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

I am deeply grateful to my major professor, Larry Halverson, for accepting me into his lab and for his advice and support throughout my degree. I am equally thankful to my co-advisor, Kirsten Hofmockel, for recruiting me to Iowa State University and for advising and encouraging me from afar. Many thanks to Michael Castellano for his advice and support in this research as well. The third chapter of this dissertation would not have been possible without help from Thomas Brutnell and Quan Zhang, who kindly provided the maize mutant and wild type maize seeds developed in their lab. I am thankful to Jessica Gutknecht for hosting me in her lab and teaching me the PLFA FAME method, as well as her contributions to the data analysis. Funding for this research was provided by the United States Department of Agriculture, Agriculture and Food Research Initiative program (grant no. 2014-67019-21628) and internal funding from the University of Minnesota. Many thanks to Guillaume Bay and Chiliang Chen for their advice and technical assistance. I also thank the undergraduate researchers Mia Bashich, Gregory Watson, Brandon Ritland, and Alyssa Nease for their efforts in sample collection and processing. Racheal Erb and Elizabeth Bach were a constant source of advice throughout my degree, for which I cannot thank them enough. Last, but certainly not least, I am deeply grateful to my significant other and my family for their love, support, and bewildering acceptance of the amount of time that I have spent (and plan to spend) in academia.

ABSTRACT

Agricultural nitrogen (N) waste is a serious problem that causes many environmental issues. Plant roots and microorganisms work in synchrony; roots exude carbon which fuels the metabolism of microorganisms, and microbes in turn increase N availability near the root that both plants and microbes can use. There is mounting interest in taking advantage of this interaction to better couple N availability with crop demand. This thesis explores questions regarding how plant roots and agricultural management interact to affect the root microbiome, with implications for soil N retention. We compared two cropping systems at the Marsden experimental farm: conventional (two-year rotation, inorganic fertilization) and diversified (four-year rotation, manure inputs). Chapter 2 investigates how maize development and agricultural management interact to shape the root-associated community. We used next-generation amplicon sequencing to assess the prokaryotic and fungal communities at four points in maize development corresponding to high or low predicted plant N demand in both cropping systems. We found that prokaryotic and fungal communities follow different patterns of assembly in relation to maize development. Additionally, roots in the diversified system hosted a bacterial community better suited to complex C decomposition at a period of high predicted N demand, implicating better coupling of N mineralization in the soil to plant demand. Chapter 3 examines competition between arbuscular mycorrhizal fungi (AMF), symbionts that help roots absorb nutrients from the soil, and ammonia-oxidizing (AO) bacteria and archaea (AOB, AOA), which mediate ammonium conversion to nitrate. Competition between these groups for ammonium may reduce AO abundance and hence nitrate leaching from soil. We used quantitative PCR to assess the abundances of AOB

and AOA in the bulk and rhizosphere soils of the conventional and diversified systems, planted with AMF deficient or AMF proficient maize genotypes. We found that the impact of AMF on the population sizes of AOB and AOA in N-rich agricultural soils is limited and that the rhizosphere and fertilization have a greater influence instead. Overall, these studies bring us closer to understanding the complex interactions between plant roots, soil management, and microorganisms that may help us build more sustainable agroecosystems with less N waste.

CHAPTER 1. GENERAL INTRODUCTION

Agricultural Sustainability

The intensification of agricultural systems in the past century has resulted in greater agricultural productivity than ever before. However, management practices such as increased fertilization, chemical inputs, and monoculture damage the environment by polluting waterways and eroding the fertile top soils that sustain us (Glendell and Brazier, 2014; Liebman et al., 2008; Pimentel and Burgess, 2013; Tomer and Liebman, 2014; Van Loo et al., 2017). Particularly problematic is nitrogen (N) use inefficiency; it is estimated that only up to 30-50% of N inputs are taken up by crops, the rest is subject to losses via conversion to gas, erosion, and leaching (Cassman et al., 2002; Hirel et al., 2011; Raun and Johnson, 1999). Agriculturally derived nitrate, the form of N that is most easily leached from soil, has caused world-wide damage to bodies of water (Carpenter et al., 1998; Vitousek et al., 1997). For instance, the Gulf of Mexico experiences seasonal hypoxia that is thousands of kilometers wide due to increases in the load of nitrate, 75% of which has been traced back to nine Midwestern states, including Iowa, and 86% of that originating from conventional corn and soybean agriculture (Alexander et al., 2008).

It is clear that modern agriculture cannot continue to operate in the same fashion as it has for the past several decades without risking environmental health. Agriculture will need to adapt new management approaches that preserve both the high-yields necessary for feeding a growing population as well as the soil health required to support that productivity (Tilman et al., 2011). The green revolution of the 1900's that spurred modern-day agriculture overlooked the ecological processes that create and maintain healthy, productive soils, and there is increasing interest to incorporate ecosystem

services back into our agriculture (Drinkwater and Snapp, 2007). Soil microorganisms, consisting of bacteria, archaea, and fungi, play important roles in soil health by building soil organic matter, stabilizing soil structure, and mediating nutrient cycling (Bender and van der Heijden, 2015; Nielsen et al., 2002). Incorporating the naturally-occurring functions of these communities into our management paradigms presents new opportunities to create more sustainable agricultural systems (Bender et al., 2016).

Root-associated microbial communities are of particular interest due to their roles in improving both plant and soil health (Berendsen et al., 2012). The rhizosphere effect promotes the growth of a specific subset of microbes near the root. Plant roots exude simple and complex carbon compounds which fuel the growth of microorganisms nearby. This stimulation allows microorganisms to increase nutrient mineralization near the root, thus feeding the plant in return (Frank and Groffman, 2009; Kraiser et al., 2011; Richardson et al., 2009). Additionally, plants have been shown to change exudation patterns that alter the expression of microbial genes associated with N cycling (Chaparro et al., 2014). As such, these plant-microbe interactions present a built-in mechanism for supplying nutrients to the plant in a way that more accurately matches demand then current methods of agricultural management, which mainly rely on the application of highly-labile inorganic N one or two times during a growing season. Despite the complex interactions between plant roots and microbial communities in the rhizosphere, many agronomic studies do not adequately consider this compartment of soil when investigating nutrient cycling and this is a large gap in need of study.

The overall goal of this research was to investigate the interactions between plants and microorganisms that affect nitrogen use efficiency in agricultural soils. To address my

goal, I conducted research at the Marsden long-term experimental site in Boone County, Iowa. This site was designed to test the hypothesis that a diversified cropping system would contribute ecosystem services that would displace the role of external inputs to maintain crop productivity (Davis et al., 2012). I considered two agricultural treatments within this experimental site for my research; conventional and diversified. The conventional treatment was designed to resemble typical corn-belt agriculture consisting of a two-crop rotation (corn, soybean) and sole reliance on inorganic fertilization. The diversified treatment, however, consisted of a four-crop rotation (corn, soybean, oat-alfalfa, alfalfa) and substituted most mineral fertilizer with composted manure. Despite a 74% reduction in synthetic N inputs, the diversified system has remained just as productive as the conventional system while decreasing nitrate leachate from the soil and increasing carbon and N retention (King and Hofmockel, 2017; Tomer and Liebman, 2014). The documented ecosystem services make this site well-suited for investigating the roles of root-associated microbial communities in agricultural sustainability.

Thesis Organization

In the second chapter of this thesis I present my work exploring the interactive effects of maize developmental stage and agricultural management on the root-associated microbial community. This study was designed to address multiple gaps in our knowledge such as 1) how prokaryotic and fungal communities respond to plant development and changing N demands in the face of differing forms of N fertilization, and 2) at what spatial scales these shifts are biologically relevant. I hypothesized that, in agricultural systems with differing forms of fertilization, the root-associated microbial community compositions would be least similar during stages of high maize N demand

due to the differences in N forms available. I also hypothesized that these effects would be strongest nearest to the root due to a higher concentration of root exudates.

In the third chapter I narrowed my focus to the interactions between the root-symbiotic fungi, arbuscular mycorrhizal fungi (AMF), and ammonia-oxidizing bacteria and archaea (AOB and AOA respectively). These groups are particularly relevant because of their roles in nutrient cycling in agricultural systems; AMF are known to aid the plant in nutrient absorption from the soil, acting as an extension of the plant root. Meanwhile, AOB and AOA mediate the first and rate-limiting step of nitrification, the conversion of ammonium to nitrate. Both of these groups are under intense scrutiny for their contrasting roles; AMF aid plant absorption and hence retention of soil nutrients, while AO promote N loss via conversion to nitrate, which is easily leached and lost from soil. I tested the hypothesis that AMF, through the ability to absorb inorganic nitrogen, would decrease the AO population size via competition for soil ammonium. I also hypothesized that these affects would differ in a cropping-system dependent manner, based on differences in ammonium availability and AMF community function.

In the final chapter, I synthesize my results from both projects and related them back to my original goal to understand the role of root-associated microbial communities in retaining N within agricultural soils. Together, these findings add to and refine our understanding of plant-microbial interactions within agricultural systems and the ecological services that they may contribute for a more sustainable future.

CHAPTER 2. MAIZE DEVELOPMENT DIFFERENTIALLY AFFECTS RHIZOPLANE PROKARYOTIC AND FUNGAL COMMUNITIES IN TWO CONTRASTING AGROECOSYSTEMS

Introduction

In modern agriculture, nitrogen (N) is one of the most important limiting resources for plant productivity and is by far the most extensively applied nutrient to our agricultural systems, with up to 80 Tg N added per year (Smil, 1999). However, applied N is generally not efficiently used by crops and is highly vulnerable to losses via conversion to gas and leaching (Cassman et al., 2002; Galloway et al., 2008). Nitrogen use inefficiency is not only economically wasteful but nitrous oxide fluxes, as a result of denitrification, and nitrate leachate, due to nitrification, contribute to greenhouse gas emissions and freshwater pollution (Carpenter et al., 1998). As an example, the Gulf of Mexico experiences a seasonal hypoxic zone thousands of miles wide due to increases in nitrate load, 75% of which has been traced back to nine Midwestern states, including Iowa, and 86% of that originating from conventional corn and soybean agriculture (Alexander et al., 2008). Agricultural management requires innovations in order to increase the coupling between N availability and crop demand if we wish to sustainably feed the human population.

Plants in unfertilized terrestrial systems mainly acquire N and other nutrients via the depolymerization of soil organic matter by microorganisms. As such, a mutualistic interaction between plant roots and microbes exists in which plants exude carbon (C) in the form of organic acids and sugars which in turn stimulate the growth and metabolic activity of microorganisms near the root (Berendsen et al., 2012; Kraiser et al., 2011; Richardson et al., 2009). As plants develop, the root-associated microbiome shifts as well

(Baudoin et al., 2002; Chiarini et al., 1998; Gomes et al., 2003; Hannula et al., 2012; Houlden et al., 2008; Wieland et al., 2001). The major mechanism by which this process occurs is thought to be via changes in root C exudation (Broeckling et al., 2008; Chaparro et al., 2014, 2013; Neumann et al., 2014; Paterson et al., 2006). In one study, the transcription of genes involved in N metabolism within the rhizosphere microbial community was altered as *Arabidopsis thaliana* aged, which also correlated to changes in root C exudates (Chaparro et al., 2014). This indicates that interactions between plant root exudation and the root microbiome are a mechanism by which the plant's nutritional needs can be met. Despite this naturally-occurring mechanism for matching plant N demand and availability, conventional agroecosystems mainly rely on external inputs of inorganic N to create a bioavailable N-supply to crops. As such, there is interest in harnessing root-associated microbial communities to develop agricultural systems with enhanced fertilizer-use efficiency (Adesemoye and Kloepper, 2009). However, we must first develop a basic understanding of how plant roots and agricultural management interact to alter the soil microbial communities that confer these ecosystem services.

The effect of agricultural management on the assembly of the root-associated microbiome is not clear. A combination pot and field experiment revealed that as inorganic N inputs increased, maize exudation patterns changed, and these changes corresponded to a shift in the microbial community of the rhizosphere (Zhu et al., 2016). Additionally, soil type can influence the trajectory of rhizosphere assembly and root exudation over the course of plant development (Chiarini et al., 1998; Hannula et al., 2012; Neumann et al., 2014; Xu et al., 2009). Together, these studies show that the environment within which a plant is grown matter for the outcome of root selection of the

microbial community. It is possible that differences in agricultural management and the forms of fertilization provided within the same soil type affect the recruitment of the root-associated microbiome, but little research has been done on this topic. Large gaps in our knowledge still exist such as how agricultural management affects rhizosphere recruitment over the course of plant development, how these changes may differ between prokaryotic and fungal communities, and at what spatial scales relative to the root that these changes are detectable in undisturbed soils.

We investigated these questions in two contrasting cropping systems within the Marsden long-term agricultural experiment; a conventional system (two-year rotation, inorganic fertilization) and a diversified system (four-year rotation, manure amendments) (Davis et al., 2012). Despite large decreases in inorganic N fertilization in the diversified cropping system, it is just as productive as the conventional system, reduces N losses to groundwater, and promotes retention of C and N (King and Hofmockel, 2017; Liebman et al., 2008; Tomer and Liebman, 2014). These documented ecosystem services allow us to use the Marsden site as a model for exploring how agricultural management affects the coupling between microbial communities and plant roots, and how this interaction may factor into increased nitrogen use efficiency in agriculture.

In this experiment, we sampled the prokaryotic and fungal communities of the maize rhizoplane (directly adhered microorganisms and soil particles), rhizosphere soil (larger soil particles clinging to the root), and bulk soil (no root influence) within the conventional and diversified systems at four points in maize development. These time points each corresponded to low, high, high, and then low predicted plant N demand (vegetative stages V4, V11, and reproductive stages R2, R5) (Licht et al., 2011). We

hypothesized that the root-associated microbial communities of maize would differ most between cropping systems at developmental stages of high-N demand (TP2 or TP3 corresponding to V11 or R2/3). This is because, if coupling between roots-and microbes is occurring, roots would select for the community most-suited to meeting nutrient demands based on the types of nutrients available and the microbial seedbank as shaped by agricultural management. In addition, we hypothesized that this effect would be stronger within the rhizoplane community compared to the rhizosphere community, due to a greater concentration of root C exudates that stimulate microbial growth in the immediate vicinity of the root.

Methods

Field Site Description

We collected soils for this experiment from the Iowa State University Marsden Long-Term Cropping System Experiment located in Boone County, IA (42°01’ N; 93°47’ W; 333 m above sea level) (Davis 2012). The Marsden site was established in 2002 and prior to that had been used for conventional corn-soybean agriculture. The site is situated on a deep, fertile Mollisol. The soil mostly comprises of Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls), Nicollet loam (fine-loamy, mixed, superactive, mesic, Aquic Hapludolls), and Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaqualls) (Davis et al., 2012). We sampled from two agricultural treatments within the Marsden site for this experiment: conventional and diversified. The conventional treatment is managed with a two-year crop rotation of corn (*Zea mays*) and soybean (*Glycine max*) with inorganic N fertilization similar to surrounding Iowan farms. The diversified treatment instead employs a four-year rotation of corn, soybean, oat/alfalfa (*Avena sativa*), alfalfa (*Medicago sativa*) and receives most of its fertilization

from composted manure and reduced inorganic fertilizer side dresses when additional N fertilization is warranted. Both systems had 90 and 60 lbs/acre UAN 32 (conventional and diversified respectively) side dress applied on 6/13/17 (management summary in Table 2.1). A more complete description and further details of farm management at the Marsden site can be found in Liebman et al. (2008). The Marsden site is set up in a completely randomized block design, and blocks 1-3 were sampled for this experiment. Each plot is 85 m x 18 m.

Sampling Protocol

After corn was seeded (05/06/16), three sampling spots were chosen randomly in each of the three replicate plots and marked. All subsequent samples were taken from within a ten-foot radius of these marks to reduce spatial heterogeneity. We chose sampling time points (TP) to best represent different phases of maize development with differing predicted N demand: TP1 (vegetative stage 4) when N demand is still relatively low, TP2 (vegetative stage 11) during rapid vegetative growth and high N uptake, TP3 (reproductive stage 2 and 3) when N uptake remains high to support kernel growth, and TP4 (reproductive stage 5) when kernel development is reaching completion and N uptake slows (Licht et al., 2011). We sampled on June 9th, June 29th, July 26th, and September 1st, 2016, corresponding to 390, 901, 1519, and 2331 growing degree days (summarized in Table S2.1). At the time of harvest, sampled corn plants were staged using the Leaf Collar method to verify developmental stage (Licht et al., 2011). If a heavy precipitation event occurred, the harvest was delayed for at least one week to reduce changes in soil moisture between sampling time points.

We collected bulk soil, rhizosphere, and the rhizoplane samples at each time point using the following protocols. Bulk soil was defined as the soil between corn rows that

did not contain roots. Three cores of the top 15 cm of soil were taken per sample halfway between corn rows and directly adjacent to the plant sampled for the rhizosphere and rhizoplane. We homogenized these bulk soil cores in surface-sterilized plastic tubs and subsampled into 2 ml centrifuge tubes and Whirl-Pak bags for DNA extraction and soil property analysis respectively. We froze all samples immediately on dry ice in the field and later stored them in a -80°C freezer.

We defined the rhizosphere as the soil still clinging to the roots after several vigorous shakes to loosen the root ball. We first used a surface-sterilized shovel to uproot the plant in the top layer (15-20 cm) of soil and shook the root ball several times to remove large and loosely-adhered soil aggregates. We placed roots into a surface-sterilized autoclave tub and cleaned off the remaining soil by further shaking and scraping with surface-sterilized tweezers and scoopulas. We used tweezers to quickly remove small roots and other visible debris from the liberated soil then transferred the soil into 2 ml centrifuge tubes and froze immediately on dry ice. At later sampling time points (TP3 and TP4), a large root-ball had developed at the base of each plant that would not loosen completely with several vigorous shakes. In this case, rhizosphere soil was only collected from roots extending from this root ball by shearing them off individually and sampling as described. After rhizosphere soil was collected, the root samples were placed into Whirl-Pak bags and frozen on dry-ice, and these were later used to isolate the rhizoplane samples. All samples were stored at -80°C.

The rhizoplane was defined as the soil particles and microbes that were still closely-associated with the root after rhizosphere sampling. For TP1 and TP2, we collected the rhizoplane from the whole root, and for TP3 and TP4, we collected the rhizoplane from

5-10 cm long healthy root segments that were equivalent in biomass to the root samples collected for TP2. Frozen root samples were placed in 40-45 ml of an ice-cold phosphate buffer (0.1 M) solution, vortexed for thirty seconds and then decanted over a 100 µm nylon mesh filter into a sterile falcon tube. Another 45 ml of ice-cold phosphate buffer solution was added to the tube with roots and then sonicated for 30 seconds at 20W using a needle probe to remove attached soil and cells. This sonicated buffer solutions were poured over another 100 µm nylon mesh and pooled with the first filtrate. The pooled filtrate was centrifuged at 10,000 RPM for 5 minutes at 4°C and then decanted, leaving a pellet of soil and root-adherent microbes. The pellet was transferred to microcentrifuge tubes and stored at -20°C before being transferred to -80°C.

Amplicon Sequencing

DNA was extracted from 0.25 g fresh weight of sample material using MoBio PowerLyzer PowerSoil kits (Mobio Laboratories, Carlsbad, CA). DNA samples were sent to Argonne National Laboratory (Lemont, IL) for amplicon library preparation and sequencing using the 16S V4/V5 region targeted by the 515F/806R primer set and the fungal internal transcribed spacer region 1 (ITS1) using the ITS1F (5’ CTTGGTCATTTAGAGGAAGTAA 3’) and ITS2 (5’ GCTGCGTTCTTCATCGATGC 3’) primer set (Smith and Peay, 2014). Amplicons of 16S and ITS were paired-end sequenced (read lengths of 250 bp x 250 bp and 150 bp x 150 bp respectively) on an Illumina MiSeq platform on separate runs. Mock community samples were also included to evaluate sequencing biases and verify taxonomic resolution. Three replicates of the ZymoBIOMICS Mock Community DNA Standard (Zymo Research, Irvine, CA) were included with 16S sequencing while three replicates of a fungal mock community were included with ITS sequencing (Bakker, in review).

Sequences were processed using the open source pipeline, hundo, available at https://github.com/pnnl/hundo. Briefly, sequences were quality filtered using BBDuk2 (Bushnell, 2014) to remove adapter and PhiX sequences at a matching kmer length of 31 bp and hamming distance of 1. At this time, all reads shorter than 51 bp were discarded. USEARCH was used to merge reads with a minimum length of 175 bp and maximum error rate of 1% (Edgar, 2010). Sequences were dereplicated and clustered using USEARCH at 97% sequence identity (distance-based, greedy clustering method) (Edgar, 2013). Chimeric sequences were predicted de novo at the same time. Taxonomic assignment to each OTU was performed using BLAST alignments (Camacho et al., 2009) and then assigned by least common ancestor across the SILVA database version 123 (Quast et al., 2013) for 16S sequences or UNITE version 7 (Kõljalg et al., 2013) for ITS sequences. We used USEARCH on seed sequences filtered against SILVA version 123 or UNITE version 7 for 16S and ITS respectively to find chimeric OTUs.

Data Analysis

All statistical analyses were performed in the software R (version 3.3). Prior to α and β diversity analyses, singleton OTUs were discarded and counts were Cumulative Sum Scaling normalized to the 75th percentile to decrease sequencing depth bias (Paulson et al., 2013). Next, data was pre-processed to remove unknown phyla, chloroplast, and mitochondrial OTUs using the 'phyloseq' package (McMurdie and Holmes, 2013). Treatment effects on richness, defined as the total number of species present in the sample, and Shannon's diversity indices were determined via Kruskal-Wallis tests using the packages 'vegan' and 'labdsv' (Oksanen et al., 2017; Roberts, 2016). Treatment effects on these α diversity metrics were tested on both raw and normalized data and

resulted in similar significance, but statistical results from the normalized data are reported here for consistency.

Distance matrices were created at the OTU-level using Bray-Curtis dissimilarity in the R packages ‘vegan’ and ‘phyloseq’ (McMurdie and Holmes, 2013; Oksanen et al., 2017). Non-metric multidimensional scaling (NMDS) ordinations were created from these distance matrices as well. Treatment effects were determined using permutational analysis of variance (PERMANOVA) with 9999 permutations. Multiple contrasts were also tested using PERMANOVA tests and a Bonferroni adjustment to determine differences in community composition between specific treatments. We also identified differentially abundant taxa between treatments using the ‘DESeq2’ package both as an additional metric of community differences, and to determine the identities of taxa differentially abundant between treatments (Anders and Huber, 2010; Love et al., 2014). DESeq2 analyzes raw data to determine the quantity and effect sizes of differentially abundant sequences between treatments and we did analyses on both OTU and family-level count data. To account for multiple comparisons, DESeq2 applies the Benjamin-Hochberg adjustment which controls the false discovery rate. We denoted significance at an α level of 0.01 to minimize family-wise error and ensure further confidence in our results. When comparing rhizoplane or rhizosphere communities between cropping systems, we filtered out differentially abundant taxa that were not also differentially abundant when compared to the corresponding bulk soil. This limited our findings to taxa that were differentially abundant within the rhizosphere and rhizoplane due to the influence of the root and not simply due to changes in environmental factors.

Mock communities were analyzed by averaging the raw counts for each OTU in the triplicate communities then iteratively searching for each known community member at each taxonomic level. Once each specific community member was found, the ratio of that member relative to the whole community was calculated and compared to the expected ratio as defined in the mock community. This provided a means for assessing sequencing biases and the accuracy of taxonomic assignment by the hundo pipeline.

Results

Prokaryotic and Fungal Community Composition

A total of 8,664,921 counts were obtained through Illumina MiSeq sequencing of the 16S region. The average number of counts per sample was 38,856.14 ± 10,440.95 (SD) counts per sample. There were a total of 19,842 unique operational taxonomic units (OTUs) identified across all samples. The most abundant phyla present in the conventional cropping system across all time points and root-proximity levels were Proteobacteria (30.7%), Acidobacteria (15.6%), Bacteroidetes (14.6%), and Verrucomicrobia (11.6%). In the diversified cropping system, the same phyla dominated the system but with differing proportions (24.5%, 15.1%, 14.9%, 14.8% respectively). Averaged across cropping systems and time points, the bulk soil community was dominated by Proteobacteria (24.5%), Acidobacteria (17.4%), Bacteroidetes (14.6%), and Verrucomicrobia (11.6%). The rhizosphere community composition was dominated by these same phyla (27.3%, 16.2%, 14.7%, 10.9% respectively), as was the rhizoplane community composition (37.9%, 22.9%, 12.4%, 10.3% respectively). Phyla-level relative abundances averaged across cropping system or root-proximity level are summarized in Figure S2.2 A and B. Phyla-level prokaryotic community compositions were also

summarized for each cropping system, time point, and root proximity treatment (Figure 2.1 A).

Illumina MiSeq sequencing of the ITS region resulted in a total of 3,635,209 counts with an average of 16,908 ± 5,967 (SD) counts per sample. The total number of unique OTUs identified across all samples was 3,136. The most abundant phyla present in the conventional cropping system across all time points and root-proximity levels were Ascomycota (59.8%), Zygomycota (22.4%), and Basidiomycota (16.6%). Within the diversified soil, these relative abundances were 52.7%, 24.8%, and 21.1% respectively. Averaged across cropping systems and time points, the bulk soil community was dominated by Ascomycota (50.7%), Zygomycota (24.3%), and Basidiomycota (16.6%). The rhizosphere community composition was dominated by these same phyla (56.7%, 21.9%, 19.9% respectively), as was the rhizoplane community composition (61.9%, 25.1%, 11.5% respectively). Phyla-level relative abundances averaged across cropping system or root-proximity level are summarized in Figure S2.2 C and D. Phyla-level fungal community compositions were also summarized for each treatment (Figure 2.1 B).

Mock Community Sequencing

Mock communities were included in sequencing to characterize bias and the taxonomic resolution of each species included (Table S2.2). Overall, the prokaryotic community was well-characterized by the 16S primers and platform used. Only one species was severely under-represented (*Lactobacillus fermentum*) and all taxa were identified within the sequences. Taxonomic assignment was correctly applied at genus level for three species, family level for three species, and order level for one species. Sequencing of the fungal mock communities returned 10 of 18 species included in the community, with three species of the same genus, *Fusarium*, undifferentiated from one

another. Of the species identified in sequencing, only one was not over or under-represented. Two species were assigned correctly at species level, four at genus level (including the three *Fusarium* species), and the remaining four at family level. Because most species in both prokaryotic and fungal communities were correctly identified at least at the family level, family-level resolution was used to evaluate the identities of differentially abundant taxa between treatments.

Treatment Effects on Diversity and Community Structure

Richness (the number of unique OTUs detected) and Shannon's diversity index were calculated to assess the α diversity of each community for each treatment. Both the richness and Shannon's diversity of the prokaryotic community were significantly influenced by time and block, but not cropping system or root-proximity ($P < 0.0001$ and $P < 0.05$ for each metric respectively). Over the course of the growing season, prokaryotic richness and Shannon's diversity decreased (Figure 2.2 A, B). Fungal community richness was significantly affected only by block ($P < 0.0001$), while fungal Shannon's diversity was significantly affected by time point and block ($P < 0.0001$ both). Fungal Shannon's diversity also generally decreased over time (Figure 2.2 C, D).

We used NMDS ordination to visualize differences in β diversity between cropping systems, root proximities, and time points. NMDS ordination showed that community composition separated clearly by root-proximity level and less clearly by cropping system and time point for both prokaryotic and fungal communities (Figure 2.3). This can be observed by the horizontal spread of treatments in differing root proximities and by the vertical spread of treatments in differing cropping systems. Using a full PERMANOVA model on the prokaryotic community data, we observed significant differences in prokaryotic community structure with highly significant main effects

(cropping system, root proximity, and time point), as well as amongst some interactions (cropping system by root proximity, and root proximity by time point, Table 2.2). Root proximity had the strongest effect on the separation of treatments followed by cropping system and time point. Block also had a significant effect (P = 0.0001) on prokaryotic community structure according to a mixed-model PERMANOVA. A full PERMANOVA model on fungal community data identified significant main effects and all significant interactions except for cropping system by root proximity by time point (Table 2.2). Root proximity had the strongest effect on community composition, followed by cropping system and time point. A mixed-model PERMANOVA revealed a significant effect of block (P = 0.0001) on fungal community structure as well.

We further explored differences in community composition between root-proximity levels within each cropping system and at each time point by comparing contrasts between specific treatments (Table 2.3). In the conventional cropping system, prokaryotic community composition in the rhizosphere soil was similar to that of the bulk soil at TP1 and TP4, but significantly differed at TP2 and TP3. The diversified system prokaryotic rhizosphere community composition was similar to the bulk soil community composition at all time points. In contrast, prokaryotic communities in the rhizoplane differed significantly in composition compared to the bulk soil communities in both cropping systems at all time points. Similarly, prokaryotic rhizosphere and rhizoplane community structures were always significantly different from one another. Comparisons of prokaryotic bulk soil communities between cropping systems at each time point revealed significant compositional differences in every case, as was the case for the rhizosphere community compositions. However, rhizoplane prokaryotic communities followed a

different pattern, and compositions significantly differed from one-another only at TP1 and TP2, and were similar at TP3 and TP4, later in plant development.

Fungal rhizosphere community composition was similar to the bulk soil community composition in the conventional system at TP1 and TP2 but not at TP3 and TP4. The fungal rhizosphere community structure in the diversified system did not differ from that of the bulk soil at TP1, TP2, and TP3 but was significantly different at TP4. However, the fungal rhizoplane community composition significantly differed from the bulk soil community composition in both cropping systems and at all time points. Similarly, the fungal rhizosphere and rhizoplane community compositions significantly differed from one another in both cropping systems at nearly all time points, except for in the conventional system at TP3. When fungal bulk soil community compositions were compared between cropping systems, they significantly differed at all time points, as did rhizosphere communities. However, Rhizoplane community structures differed significantly between cropping systems only at TP1 and TP4.

The interaction of root proximity and time point for both prokaryotic and fungal communities were both highly significant and had similar R^2 values as the main effect of cropping system, indicating that this interaction may be important for understanding the spatial and temporal dynamics of these communities. In order to explore this interaction, further contrasts were conducted between communities between consecutive time points for each cropping system and at each root proximity level (Table 2.4). Additionally, the NMDS ordination of the data was faceted to visualize these changes over time (Figure 2.4). Prokaryotic bulk soil and rhizosphere soil communities followed similar trends and did not differ from one another within either cropping system over the entire growing

season. In contrast, the rhizoplane prokaryotic community compositions differed significantly between all time points within both cropping systems. Similar to the prokaryotic communities, fungal bulk soil community compositions did not significantly differ from one another within either cropping system and at any time point comparisons. In the rhizosphere, fungal communities differed from one another within the conventional system between TP1 and TP2 and between TP2 and TP3, and within the diversified system between TP2 and TP3, and between TP3 and TP4, showing more temporal shifts than the prokaryotic rhizosphere communities. Fungal rhizoplane communities significantly differed from one another between all time points except for within the conventional system between TP2 and TP3 and within the diversified system between TP1 and TP2.

Differentially Abundant Taxa

In order to further assess dissimilarity between communities of different cropping systems at each root proximity level, differential abundance analyses were conducted on communities at the OTU and family levels using DESeq2. Differential abundance at the OTU level was used to quantify the number of differences between treatments while family-level differences were used to assess the identity of taxa constituting those differences. The family level was chosen due to the degree of taxonomic resolution we could rely on given the sequencing results of the mock communities (Table S2.1). The OTU-level differential abundance results informed the family-level analyses, and rhizoplane communities were chosen as the focus of these further analyses because of the greater differences between rhizoplane and bulk soil communities as compared to bulk soil vs rhizosphere soil communities.

At the OTU level, the greatest number of differentially abundant prokaryotic taxa between bulk soil communities occurred at TP3, while the rhizosphere and rhizoplane peaked at TP2 and decreased thereafter (Figure 2.5 A, Table S2.3). Because taxa that were not differentially abundant compared to the bulk soil were filtered from rhizosphere and rhizoplane differential abundance contrasts, the rhizosphere communities had many fewer OTUs differentiating them between cropping systems compared to those of the rhizoplane due to the greater similarity between bulk and rhizosphere soil communities. At TP2, when the most taxa differences were observed between rhizoplanes in the two cropping systems, the three families that were most abundant in the diversified compared to the conventional cropping system rhizoplane were Glycomycetaceae, an unknown family within Acidobacteria Subgroup 18, and Saprospiraceae (Figure. 2.6 A). Families most abundant in the conventional rhizoplane compared to the diversified rhizoplane were the Catenulisporaceae, Actinobacteria 288-2, and Burkholderiaceae (Figure. 2.6 A).

Bulk soil, rhizosphere soil, and rhizoplane fungal communities had the most differentially abundant OTUs between cropping systems at TP1 (Figure 2.5 B, Table S2.3). At TP1 the diversified cropping system families that were most abundant compared to the conventional cropping system were unknown families within the Saccharomycetes, Helotiales, and Pleosporales (Figure 2.6 B). The top three fungal families most abundant in the conventional compared to the diversified rhizoplane were an unknown family within the Sacharomycetales, Helotiaceae, and Mycosphaerellaceae (Figure 2.6 B). Overall, majority of the differentially abundant families between cropping systems belonged to the phylum Ascomycota (13 of 23 families detected).

Discussion

Though many studies have investigated how the root-associated community changes as the plant develops, we are unaware of any that have directly compared the impacts of differing management systems on this process in the field. We investigated the effects of a diversified (four-year rotation, manure amendments) and conventional (two-year rotation, inorganic fertilization) maize cropping system on the dynamics of prokaryotic and fungal root communities through the course of maize development as plant N demands change. Our results demonstrate that root-associated prokaryotic communities differed most between cropping systems at a developmental stage consistent with high plant N uptake and became more similar thereafter. Prokaryotic groups associated with organic matter decomposition were enriched at the high N demand stage within the diversified rhizoplane community. Root-associated fungal communities, however, appear to differ most between cropping systems early in plant development and then converged. Additionally, rhizoplane communities were much more responsive to root selection compared to rhizosphere communities, making the patterns of assembly clearest within the rhizoplane.

Surprisingly, we did not find significant shifts in community richness or Shannon's diversity due to cropping system or root-proximity, but instead saw a significant decrease in α diversity measurements over the course of the growing season. The effects of agricultural management on microbial diversity are often inconsistent, with some studies indicating increased diversity within organic-matter fed agricultural systems and others reporting a decrease or no difference (Cesarano et al., 2017; Fernandez et al., 2016; Hartmann et al., 2015; Kamaa et al., 2011; Sugiyama et al., 2010; Zhong et al., 2010). Environmental factors may contribute to the overall decline in richness and diversity over

time in these systems, such as the addition of a fertilizer side-dress prior to TP2 that was applied to both cropping systems in this study. Soil N deposition has been shown to decrease microbial α diversity in the overall community or of specific microbial groups (Campbell et al., 2010; Li et al., 2017; Zeng et al., 2016).

Overall, root-associated communities, particularly within the rhizoplane, shifted significantly over the course of plant development, indicating root-selection of particular microbial groups. These results are consistent with those of other studies that have investigated the effect of plant development on the root-associated community structure, which document that different plant developmental stages select for different microbial communities (Chiarini et al., 1998; Gomes et al., 2003; Hannula et al., 2012; Houlden et al., 2008; Micallef et al., 2009; Xu et al., 2009). These shifts in community composition are likely caused by changes in root-exudation during growth which has been demonstrated for both prokaryotic and fungal communities in various plant species (Broeckling et al., 2008; Chaparro et al., 2014, 2013; Paterson et al., 2006). Our first hypothesis, that root-associated microbial communities would differ most between cropping systems at stages of rapid plant growth and high N demand, was partially supported. Prokaryotic rhizoplane communities showed the least overlap in community composition and the most differentially abundant taxa at TP2, when maize plants are known to be growing rapidly with high N uptake (Licht et al., 2011). To our knowledge, this experiment presents the first evidence that agricultural management can have a marked-impact on the trajectory of the prokaryotic community structure as the plant ages, and that the magnitude of this difference varies by plant developmental stage.

Families within the Verrucomicrobia and Acidobacteria were commonly and consistently more abundant in the diversified rhizoplane compared to the conventional rhizoplane at TP2, when maize were at a stage of high nutrient demand and displayed the most differences between rhizoplane communities. Meta-analyses have provided evidence for the ecological roles of these phyla as oligotrophs important for the breakdown of complex organic materials and C cycling in soil systems (Fierer et al., 2013, 2007). In support of this, whole genome-analyses of culturable members of the Verrucomicrobia and Acidobacteria identified gene pathways suited for the breakdown of complex, plant-derived polysaccharides (Martinez-Garcia et al., 2012; Ward et al., 2009). Additionally, members of both phyla are capable of rhizosphere colonization and have been isolated from the plant root surface (Nunes da Rocha et al., 2013, 2011; Tanaka et al., 2017). Considered together, this evidence supports the notion that bacteria may play a key role in the diversified system by decomposing more complex organic material near the root, thus liberating nutrients to support plant growth. In juxtaposition to this, within the conventional system, families of the Proteobacteria and Actinobacteria comprised the majority of taxa enriched in the rhizoplane compared to in the diversified system, which are considered fast-growing copiotrophs (Fierer et al., 2007). Proteobacteria and Actinobacteria have been consistently identified as rapid consumers of simple C substrates, unlike Verrucomicrobia that participate in the decomposition of more complex plant-derived C (Kramer et al., 2016; Pepe-Ranney et al., 2016). Therefore, the conventional rhizoplane hosts comparatively higher abundances of prokaryotes suited to simple C substrates, and lower abundances of prokaryotes suited to complex C decomposition, indicating a higher reliance on inorganic inputs that are readily available.

Management that relies exclusively on inorganic inputs may select for microbial communities dependent on easily-accessed nutrition, and disrupt the plant's ability to select for a community that mineralizes nutrients from organic matter near the root.

The convergence of rhizoplane communities between cropping systems at TP3 and TP4 (corresponding to high and low predicted N demand respectively) may indicate that the roots in the two systems selected for similar communities at these stages. Otherwise, the types of roots selected for sampling at TP3 and TP4 may have influenced the results, as at those time points only matured roots would be present in the top-layer of soil sampled. Mature roots have been shown to shape root-associated prokaryotes differently compared to developing roots (DeAngelis et al., 2009).

Fungal root-associated communities of differing cropping systems were most different from one another at TP1, when maize growth and N demand were not predicted to be high compared to other stages of plant development (Licht et al., 2011). In this case, fungal communities did not appear to conform to the nutrient demands and specific forms of nutrients available to the plant as did prokaryotic communities. At TP1, when fungal rhizoplane communities were most distinct between cropping systems, the majority of differentially abundant taxa were families within the Ascomycota, which supports the findings of Gomes et al. (2013) who found that two maize cultivars consistently selected for various groups within the Ascomycota at early stages of development. The order Pleosporales was found to be consistently selected for early in plant development in Gomes et al. (2013) and was also present within TP1 rhizoplanes of maize in this experiment, with higher abundance within the diversified-grown maize rhizoplane. Species of Pleosporales and Ascomycota in general cover a broad-range of ecological

roles, such as saprotrophs, root endophytes, and plant pathogens (Schoch et al., 2009; Y. Zhang et al., 2012; Zhang et al., 2009). Though at the family-level it is impossible to determine the functional roles of the taxa detected in this study due to the limited information available, it is possible that Ascomycota are early colonizers of maize within agricultural systems, and that the fungal seedbank as shaped by agricultural management affects the early recruitment of Ascomycota by maize roots. This is supported by data showing that Ascomycota comprised the majority of differentially abundant taxa in the bulk soils of these systems as well (Figure S2.3). Culturable Ascomycota within the families identified here make excellent candidates for further examination of colonization strategies, contributions to plant health, and their roles within the rhizosphere in plant development.

Our second hypothesis, that root selection within the root-associated community would be stronger in the rhizoplane than within the rhizosphere, was supported. Though this finding was predicted, the lack of differentiation between rhizosphere and bulk soil communities within the prokaryotic data is surprising, as root exudates have been found to reach up to several mm from the root surface within soils and to effect microbial communities up to 5 mm from the root in controlled studies (Kandeler et al., 2002; Kuzyakov et al., 2003; Raynaud, 2010). In contrast, our experiment was conducted under field conditions with spatially heterogeneous soils and variable moisture conditions, which may have limited the spread of root exudates into the rhizosphere. In contrast, many field-conducted experiments investigating the rhizosphere soil report a significant change in community structure compared to bulk soils (Hargreaves et al., 2015; Fernandez et al., 2016; Yin et al., 2017; Yurgel et al., 2017). However, many of these

experiments likely include at least part of the rhizoplane communities in the washing steps used to collect the rhizosphere samples. This may indicate that methods to isolate the root-associated community that do not employ a wash, vortex, or sonication step may not adequately distinguish bulk and root-affected soils. It is possible that the rhizoplane is a larger driver of the rhizosphere effect under field conditions than previously thought. This is in accordance with the theoretical work by Watt et al. (2006) who predicted that the rhizoplane community would be much more responsive to root exudation due to its close proximity to the root and the heterogeneity of the soil under field conditions. This, taken together with the observation that many of the changes in rhizoplane community structure corresponding to plant development were not also seen in the rhizosphere, indicates that field experiments should take special care to include the rhizoplane in all "rhizosphere" sampling efforts, lest they miss important dynamics of the root-associated community.

Fungal communities responded to root selection further from the root compared to prokaryotic communities. The bulk soil microbial community structure remained largely stable over the course of the growing season but fungal community structure significantly shifted within both the rhizoplane and rhizosphere between sampling time points, while only rhizoplane prokaryotic communities responded to plant development. Most bacteria and archaea are limited in their spread to water-filled volumes within soils, whereas fungal hyphae are well-suited to exploring heterogeneous soil environments (Pajor et al., 2010). Various experiments on root-colonizing symbiotic and pathogenic fungi have found that hyphae can spread up to several centimeters through the soil from the root (Friese and Allen, 1991; Otten et al., 2004; Otten and Gilligan, 1998). Fungi closely

associated with plant roots, therefore, would easily spread to accommodate the rhizosphere, unlike prokaryotes. The spatial scales at which prokaryotes and fungi may be affected by plant growth appear to differ and should be considered in future studies of microbial function within agricultural systems.

Conclusions

In conclusion, this multi-faceted experiment contributes to our understanding of how agricultural management and root selection interact to shape root-associated microbial communities within differing agricultural systems. We show that during developmental stages of fast growth and high nutrient demand, maize roots in differing cropping systems selected for dissimilar prokaryotic communities. In a manure-fed cropping system, the prokaryotic rhizoplane community included a higher abundance of members implicated in complex organic matter decomposition, while in a conventional, inorganically fed cropping system, the rhizoplane communities contained a higher abundance of taxa reliant on more easily-accessed nutrition. This may be one mechanism by which the diversified system is able to support high productivity with reduced fertilization. In contrast, root-associated fungal communities appeared to be selected first based on the fungal seed bank supplied by the management regime and then converged to be highly similar regardless of agricultural management or predicted plant N demand. This indicates that management may have important effects on the root-associated fungal community early in plant development. Finally, we provide evidence that rhizoplane communities may be a main driver of the differences between bulk and rhizosphere soil, particularly for prokaryotic communities, and should not be excluded in field sampling efforts. This close association may mean that plant-growth promoting properties of the

root-associated community occur at a much more intimate scale relative to the root than previously expected, particularly in undisturbed soils.

Future studies should investigate how differences in fertilization affect plant exudation patterns to see if shifts in plant exudation may account for the changes in root-associated microbial communities. Additionally, the functional capacity of the root-associated microbial communities, and not just taxa identities, should be explored to understand how shifts in plant development in differing agricultural systems affect soil functioning. In order to fully utilize the ecological services conferred by plant-microbe interactions in our agricultural systems, we must continue to unravel the spatio-temporal dynamics of prokaryotic and fungal communities for targeted management decisions.

Supplementary Methods

Bulk soil chemical properties were also measured at the time of sampling. A 25-50 g subsample of bulk soil was placed into a Whirl-Pak bag and frozen immediately on dry ice, then transferred to a -80C freezer. Soil chemical properties were evaluated by the Iowa State University Plant and Soil Analysis Laboratory using their standard protocols (Eliason et al., 2015; Kalra, 1998) (Table S2.4). Briefly, soil NH_4^+ and NO_3^- concentrations were determined colorimetrically and phosphorus, potassium, calcium, and iron were all determined via Mehlich-3 extraction read on an ICP. The soil pH was measured via electrode in a soil and water slurry. Gravimetric water content was measured via drying fresh soil in an oven for 48 hours. Total N and C were measured via combustion analyses. Significance of main effects were determined via multi-way ANOVA (Table S2.5).

Table 2.1. Agricultural management of the Marsden site during the 2016 growing season and previous years. N/A means that no treatment was applied in that. UAN 32 refers to the fertilizer type used (solution of 45% ammonium nitrate, 35% urea, and 20% water).

Cropping System	Rotation (Year)	Fertilization	Side Dress
Conventional	Corn (2016)	100 lbs/acre UAN 32 (at planting)	90 lbs/acre UAN 32 (6/13/17)
	Soybean (2015)	N/A	N/A
Diversified	Corn (2016)	7.6 tons/acre composted manure (Nov 2015)	60 lb/acre UAN 32 (6/13/17)
	Alfalfa (2015)	N/A	N/A
	Oat and alfalfa (2014)	N/A	N/A
	Soybean (2013)	N/A	N/A

Table 2.2. Permutational analysis of variance of β diversity. Results are based on 9999 permutations.

	Prokaryotic		Fungal	
Treatment Effect	R^2	**P-value**	R^2	**P-value**
Cropping system	0.065	0.0001	0.083	0.0001
Root proximity	0.278	0.0001	0.166	0.0001
Time point	0.056	0.0001	0.095	0.0001
Cropping system * Root proximity	0.015	0.0011	0.018	0.0001
Cropping system * Time point	0.010	0.0943	0.022	0.0001
Root proximity * Time point	0.059	0.0001	0.066	0.0001
Cropping System * Root proximity * Time point	0.014	0.5692	0.019	0.1790

Table 2.3. Significance of β diversity between root-proximities. Results are based on multiple PERMANOVA tests (9999 permutations) with Bonferroni adjustment. Numbers represent P-values. Contrasts that compare the same root-proximity level do so between cropping systems. Conv. and Div. refer to conventional and diversified cropping system respectively.

	Time point	TP1		TP2		TP3		TP4	
	Cropping System	Conv.	Div.	Conv.	Div.	Conv.	Div.	Conv.	Div.
	Contrasts								
Prokaryotic	Bulk vs Rhizosphere	0.313	1.000	0.038*	0.464	0.011*	1.000	0.200	1.000
	Bulk vs Rhizoplane	0.027*	0.005**	0.027*	0.027*	0.022*	0.027*	0.027*	0.027*
	Rhizosphere vs Rhizoplane	0.027*	0.005**	0.027*	0.027*	0.033*	0.027*	0.027*	0.027*
	Bulk vs Bulk	0.027*		0.027*		0.049*		0.027*	
	Rhizosphere vs Rhizosphere	0.027*		0.027*		0.049*		0.027*	
	Rhizoplane vs Rhizoplane	0.005*		0.027*		0.059		0.162	
Fungal	Bulk vs Rhizosphere	0.173	0.270	0.124	1.000	0.011*	0.335	0.044*	0.027*
	Bulk vs Rhizoplane	0.005**	0.022*	0.038*	0.043*	0.032*	0.027*	0.027*	0.043*
	Rhizosphere vs Rhizoplane	0.005**	0.022*	0.011*	0.043*	0.086	0.027*	0.027*	0.043*
	Bulk vs Bulk	0.027*		0.016*		0.027*		0.027*	
	Rhizosphere vs Rhizosphere	0.027*		0.027*		0.011*		0.027*	
	Rhizoplane vs Rhizoplane	0.032*		0.113		0.070		0.043*	

Table 2.4. Significance of β diversity between consecutive time points. Results are based on multiple PERMANOVA tests (9999 permutations) with Bonferroni adjustment. Numbers represent P-values. Conv. and Div. refer to the conventional and diversified cropping systems respectively.

	Root proximity	Bulk		Rhizosphere		Rhizoplane	
	Cropping system	Conv.	Div.	Conv.	Div.	Conv.	Div.
	Contrasts						
Prokaryotic	TP1 vs TP2	0.162	1.000	0.119	0.113	0.027*	0.005*
	TP2 vs TP3	0.826	1.000	0.497	0.956	0.022*	0.027*
	TP3 vs TP4	1.000	1.000	1.000	1.000	0.016*	0.027*
Fungal	TP1 vs TP2	1.000	0.319	0.043*	0.130	0.016*	0.065
	TP2 vs TP3	0.329	1.000	0.027*	0.027*	0.081	0.027*
	TP3 vs TP4	1.000	1.000	0.259	0.027*	0.005**	0.043*

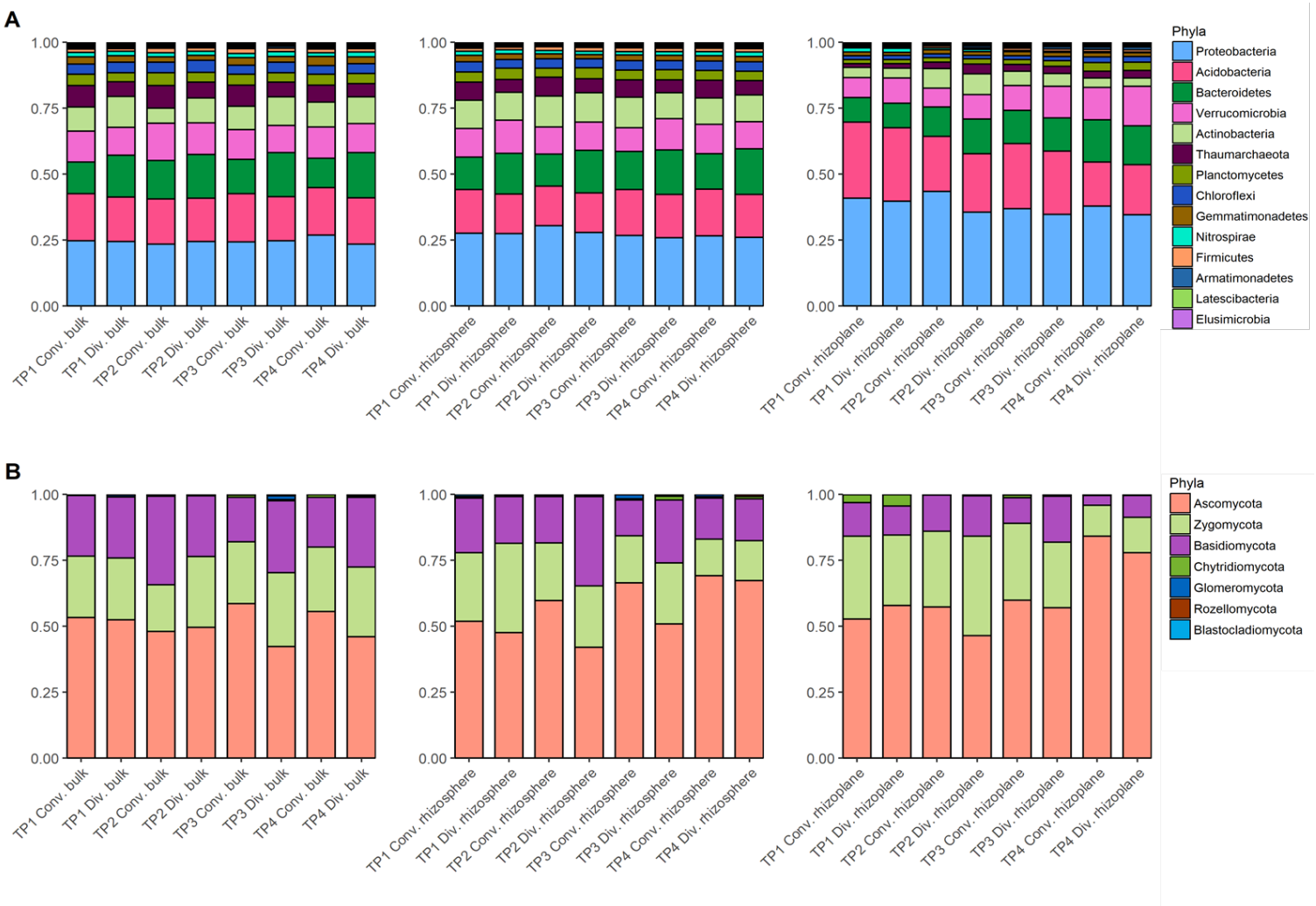

Figure 2.1. Relative abundance of phyla in each treatment. **A)** Dominant prokaryotic phyla and **B)** dominant fungal phyla. Low abundance prokaryotic phyla not visible in the graph (total of 54 phyla) are not shown in the legend while all fungal phyla detected are present in the legend (total of 7 phyla).

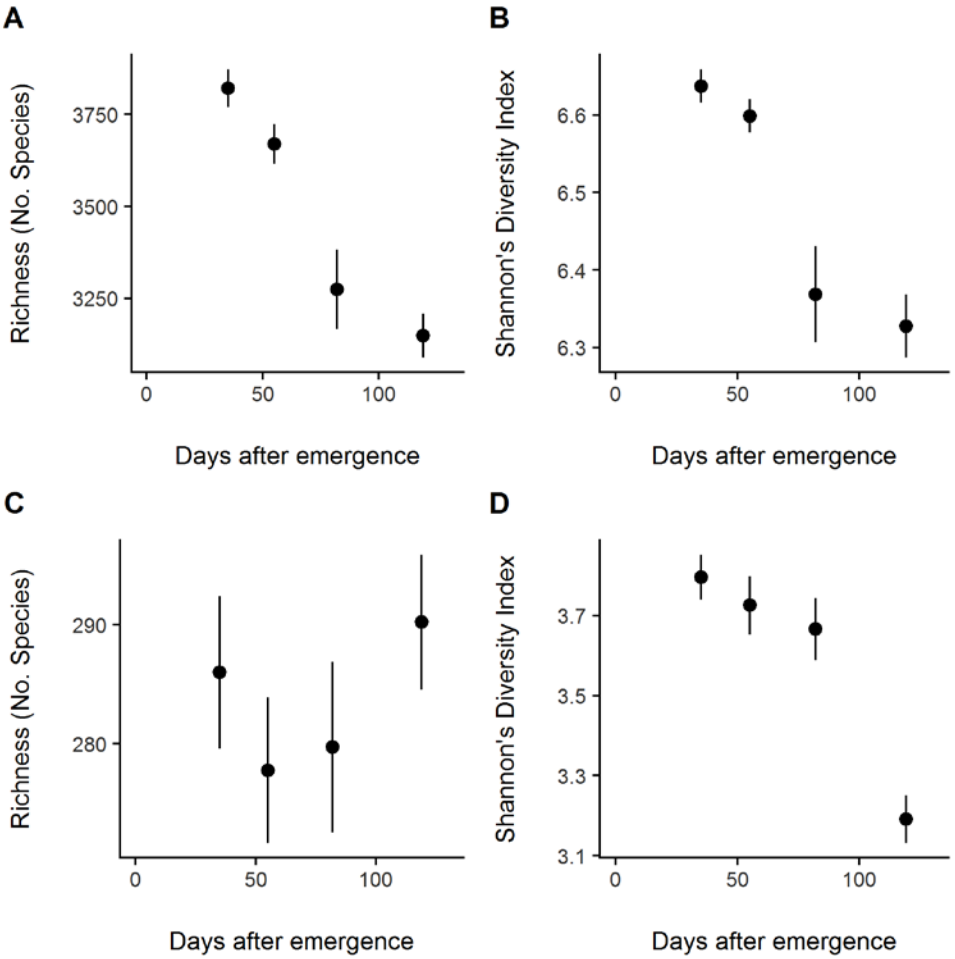

Figure 2.2. Richness and Shannon's Diversity over time. **A-B)** prokaryotic and **C-D)** fungal communities averaged over cropping system and root-proximity level. Points indicate means and bars indicate standard error. The main effect of time was statistically significant according to Kruskal-Wallis test ($P < 0.05$) for all except fungal richness.

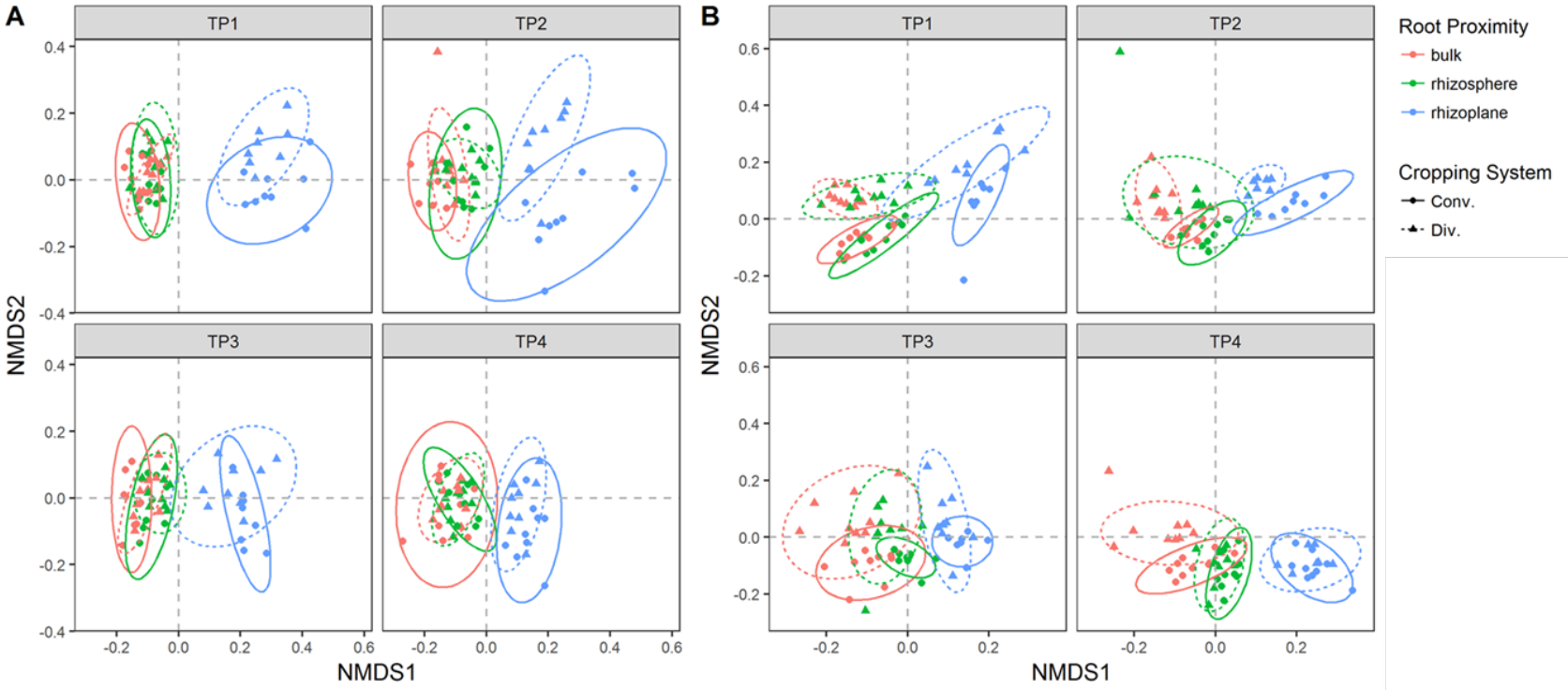

Figure 2.3. NMDS ordinations of microbial communities. Ordinations are based on the Bray-Curtis dissimilarities of CSS normalized data. **A)** Prokaryotic, stress = 0.142, and **B)** fungal, stress = 0.201, communities of each treatment. Ellipses indicate T-normal distributions of each treatment group.

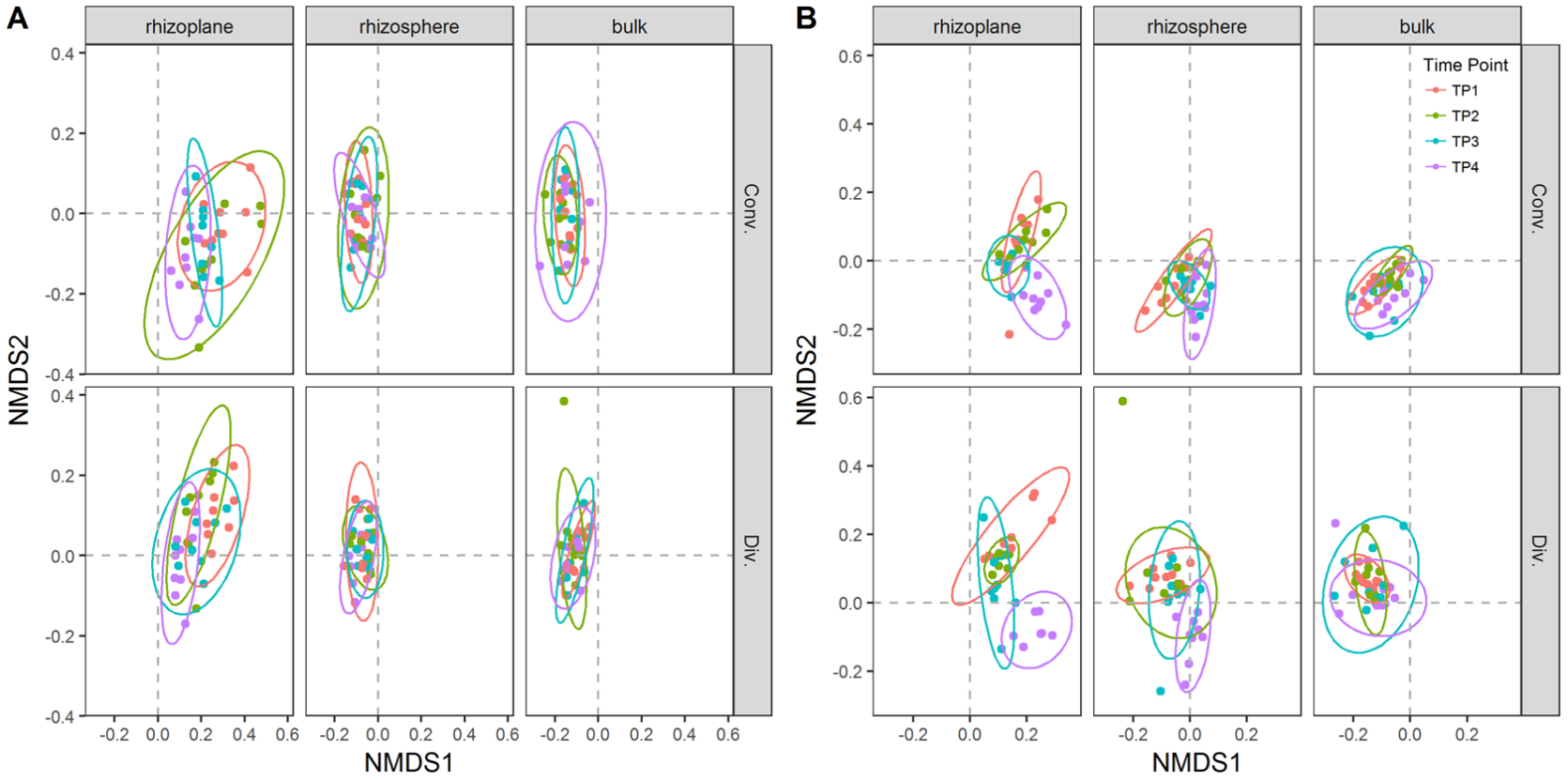

Figure 2.4. NMDS ordinations of root proximity and time point interactions. **A)** Prokaryotic, stress = 0.142, and **B)** fungal, stress = 0.201, communities faceted by root-proximity and cropping system. Ellipses show the t-normal distribution of each treatment group.

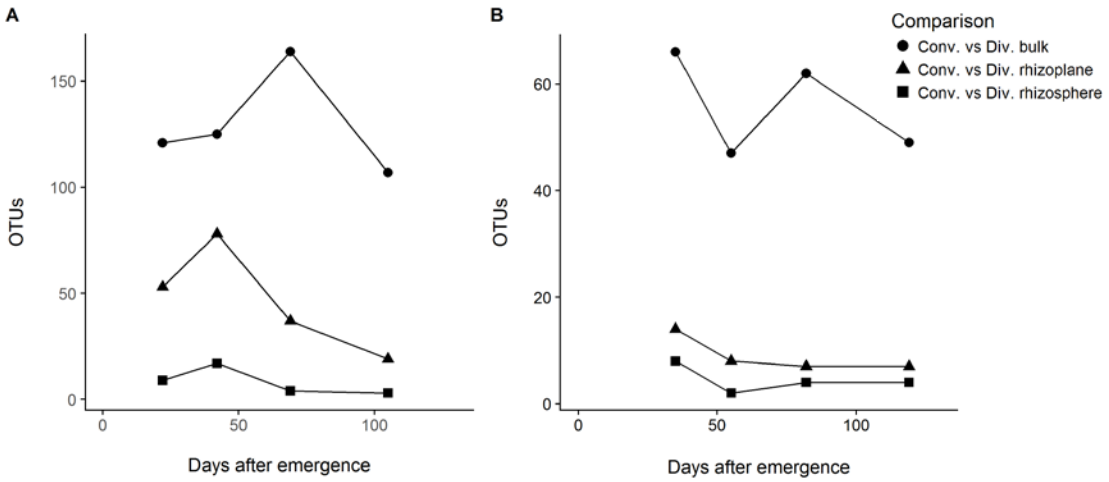

Figure 2.5. Number of differentially abundant OTUs between cropping systems in the bulk soil, rhizosphere soil, and rhizoplane. **A)** Prokaryotic and **B)** fungal communities at each time point.

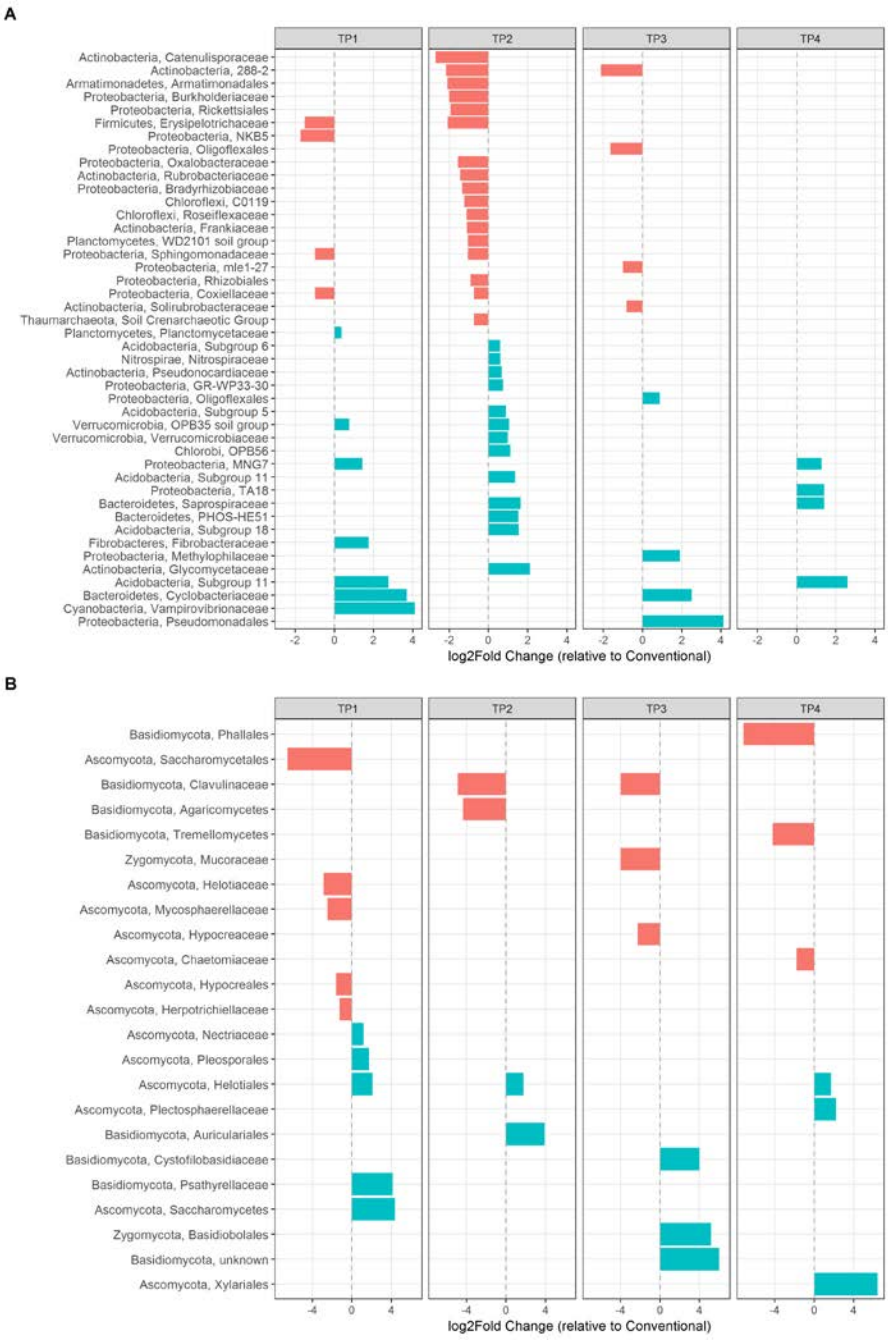

Figure 2.6. Family-level differential abundance between rhizoplanes of differing cropping systems at each time point. **A)** Prokaryotic and **B)** fungal communities. The y-axis shows the phylum and family assignment of each taxa. If the family was unknown, the class or order assignment was substituted. A red bar indicates a lower abundance of the taxa in the diversified system, while a blue bar indicates a higher abundance of the taxa in the diversified system. A log2Fold Change of 1.5 indicates that the taxa was $2^{1.5}$ times the abundance of the conventional system.

Table S2.1. Summary of maize properties at sampling time points.

Time point	TP1	TP2	TP3	TP4
Developmental Stage	V4	V11	R2	R5
Growing Degree Days	390	901	1519	2331
Calendar Date	6/9/2016	6/29/2017	7/26/2016	9/1/2016
Predicted N Demand	Low	High	High	Low

Table S2.2. Mock community sequencing results.

Prokaryotic

Species	**OTU ID**	**Theoretical Ratio**	**Actual Ratio**	**Assigned Correctly**
Pseudomonas aeruginosa	OTU_155	4.6	6.1	Order
Escherichia coli	OTU_119	10	7.0	Family
Salmonella enterica	OTU_9	11.3	11.2	Family
Lactobacillus fermentum	OTU_170	18.8	8.5	Family
Enterococcus faecalis	OTU_80	10.4	11.7	Genus
Staphylococcus aureus	OTU_39	13.3	20.9	Genus
Listeria monocytogenes	OTU_44	15.9	17.9	Genus
Bacillus subtilis	OTU_61	15.7	16.6	Genus

Fungal

Species	**OTU ID**	**Theoretical Ratio**	**Actual Ratio**	**Assigned Correctly**
Alternia alternata	OTU_12	0.0263	0.126	Species
Aspergillus flavus	N/A	0.263	0	Not Found
Neosartorya fischeri	N/A	0.263	0	Not Found
Penicillium expansum	N/A	0.263	0	Not Found
Candida apicola	N/A	0.263	0	Not Found
Saccharomyces cerevisiae	OTU_580	2.63	0.858	Genus
Claviceps purpurea	OTU_1141	0.263	0.062	Family
Trichoderma reesei	OTU_433	0.263	0.031	Family
Fusarium graminearum, oxysporum, and vesticilloides	OTU_119, OTU_3	16.04	24.151	Genus
Soitoella complicata	OTU_58	52.6	19.102	Species
Rhizoctonia solani	OTU_900	0.132	0.140	Family
Naganishia albida	OTU_21	26.3	54.763	Genus
Chytriomyces hyalinus	N/A	0.263	0	Not Found
Rhizophagus irregularis	N/A	0.0263	0	Not Found
Mortierella verticillata	OTU_427	0.263	0.758	Genus
Rhizomucor miehei	OTU_3005	0.132	0.008	Family

Table S2.3. Differentially abundant OTUs between cropping systems.

	Time Point	Bulk	Rhizosphere	Rhizoplane
Prokaryotic	TP1	121	9	53
	TP2	125	17	78
	TP3	164	4	37
	TP4	107	3	19
Fungal	TP1	66	8	14
	TP2	47	2	8
	TP3	62	4	7
	TP4	49	4	7

Table S2.4. Bulk soil chemical properties at each time point. Numbers report means and standard errors (n=9). GWC refers to gravimetric water content.

Cropping System	Time Point	Total C (%)	Total N (%)	Ammonium (mg/kg)	Nitrate (mg/kg)	Phosphorus (mg/kg)	Potassium (mg/kg)	Iron (mg/kg)	pH	GWC
Conventional	**TP1**	2.5 ± 0.1	0.23 ± 0.03	4.6 ± 0.4	25.7 ± 2.1	30.9 ± 3.3	227.4 ± 17.0	153.6 ± 10.6	6.3 ± 0.1	0.22 ± 0.03
	TP2	2.4 ± 0.1	0.24 ± 0.02	52.7 ± 20.9	29.7 ± 6.8	33.7 ± 3.9	242.7 ± 16.8	164.8 ± 13.0	6.5 ± 0.2	0.12 ± 0.004
	TP3	2.4 ± 0.1	0.23 ± 0.03	51.3 ± 23.3	38.4 ± 11.6	33.2 ± 4.8	191.9 ± 14.7	163.3 ± 9.5	6.5 ± 0.2	0.19 ± 0.004
	TP4	2.5 ± 0.1	0.22 ± 0.03	8.4 ± 2.7	12.7 ± 4.6	31.6 ± 3.6	234.3 ± 22.3	173.9 ± 13.5	6.4 ± 0.1	0.18 ± 0.02
Diversified	**TP1**	2.4 ± 0.1	0.23 ± 0.01	3.2 ± 0.3	14.7 ± 2	39.2 ± 5.6	179.4 ± 12	162.4 ± 11.2	6.9 ± 0.1	0.18 ± 0.005
	TP2	2.3 ± 0.1	0.24 ± 0.01	73 ± 54.5	33.4 ± 18.7	44.9 ± 6.6	175.0 ± 12.5	170.7 ± 11.4	6.9 ± 0.1	0.13 ± 0.005
	TP3	2.4 ± 0.1	0.23 ± 0.01	17.9 ± 11.1	24 ± 7.7	38.2 ± 4.1	144.0 ± 9.0	169.9 ± 10.2	6.8 ± 0.1	0.21 ± 0.005
	TP4	2.4 ± 0.1	0.21 ± 0.01	7.3 ± 1.9	9.8 ± 1.7	35.2 ± 5.1	133.4 ± 6.5	178.0 ± 11.1	6.7 ± 0.1	0.22 ± 0.02

Table S2.5. Statistical significance of bulk soil properties. Significance was determined via mixed-model ANOVA. Numbers represent P-values. GWC refers to gravimetric water content.

Soil Poperty	Cropping System	Time point	Soil*Timepoint	Block
Total C	0.154	0.922	0.976	0.001
Total N	0.308	0.032	0.953	0.109
Ammonium	0.093	< 0.001	0.823	0.871
Nitrate	0.440	< 0.001	0.541	0.952
Phosphorus	0.035	0.633	0.846	0.069
Potassium	< 0.001	0.023	0.220	0.122
Iron	0.387	0.393	0.997	0.001
pH	0.033	0.617	0.822	0.018
GWC	0.685	< 0.001	0.015	0.248

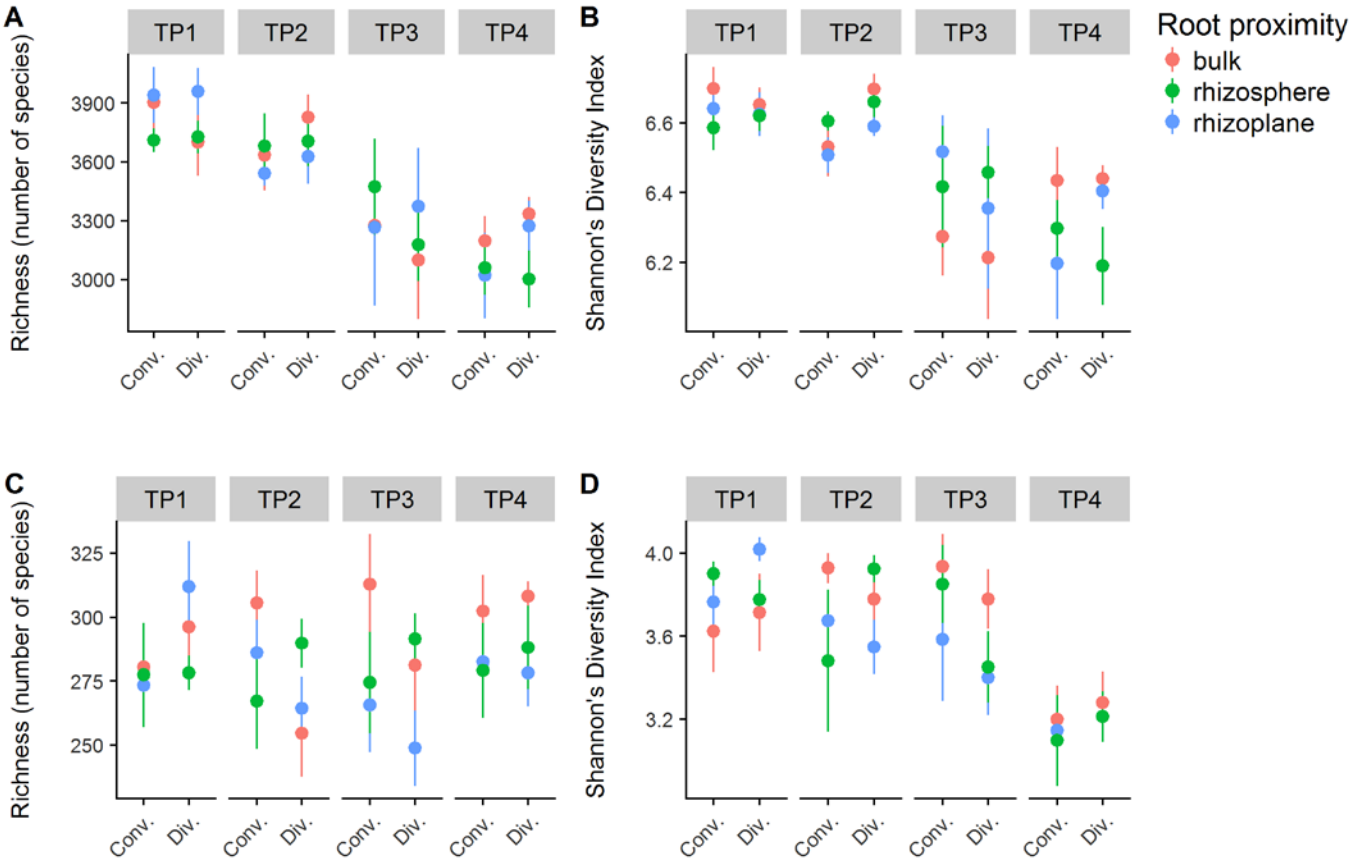

Figure S2.1. α diversity of prokaryotic and fungal communities over maize development in two agricultural systems. **A and B)** Prokaryotic richness and Shannon's Diversity Index, and **C and D)** fungal richness and Shannon's Diversity Index. Points indicate means and bars indicate standard error.

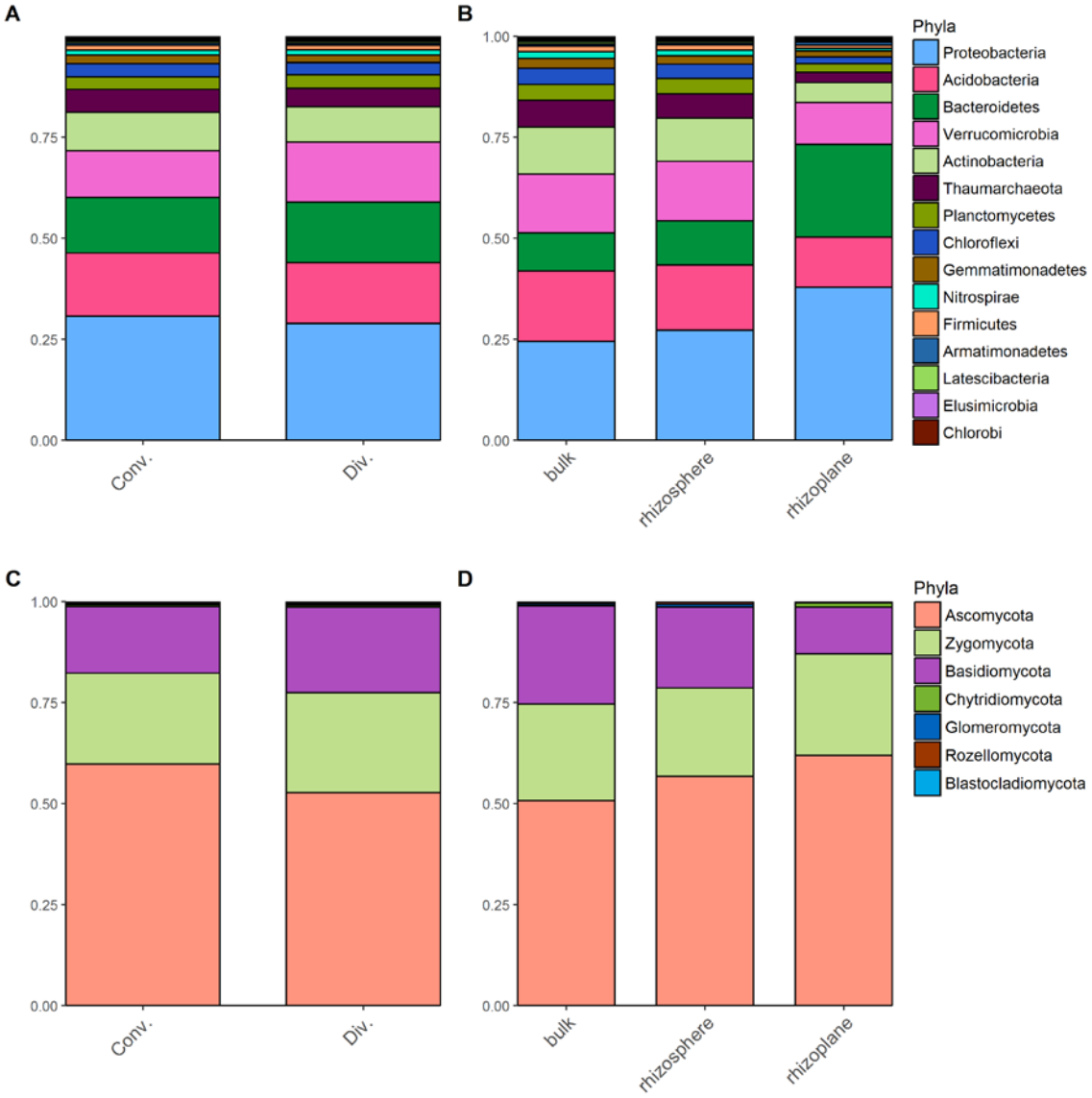

Figure S2.2. Averaged relative abundance of prokaryotic and fungal phyla. **A and B)** Prokaryotic phyla averaged over cropping system and root proximity, and **C and D)** fungal phyla averaged over cropping system and root proximity. Conv. and Div. refer to the conventional and diversified systems respectively.

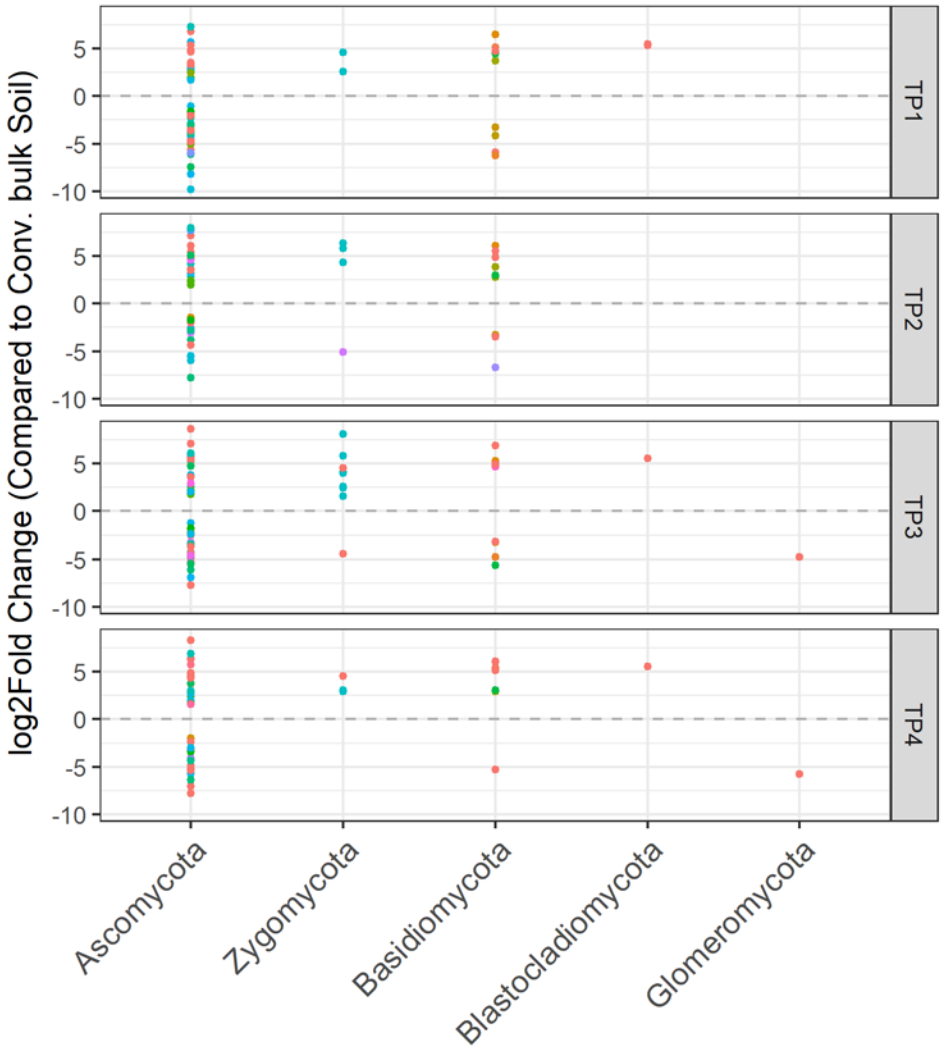

Figure S2.3. Differential abundance of fungal OTUs between bulk soils of the conventional and diversified cropping systems. Each point represents a unique OTU, colored by family (legend not provided due to length). A log2Fold Change of 1.5 indicates that the taxa was $2^{1.5}$ times the abundance of the conventional system.

CHAPTER 3. THE RHIZOSPHERE AND SOIL MANAGEMENT, BUT NOT ARBUSCULAR MYCORRHIZAE, EFFECT AMMONIA-OXIDIZING ARCHAEA AND BACTERIA ABUNDANCES IN TWO AGRICULTURAL SOILS

Introduction

Ammonia oxidizers (AO), comprised of bacteria (AOB) and archaea (AOA), play an important role in the nitrogen (N) cycle. AO mediate the first step of nitrification, the process which converts ammonium (NH_4^+) into nitrate (NO_3^-). Nitrification has become a key issue in agricultural soils where ammonium and urea fertilizers are often applied in excess, or in asynchronous timing, of what crops are able to use (FAO, 2009). The NO_3^- resulting from nitrification is highly labile and easily leached into waterways where it can cause hypoxia and ecological damage, such as the growing "dead-zone" of the Gulf of Mexico which has grown to be thousands of miles wide (Burkart and James, 1999; Carpenter et al., 1998; Di and Cameron, 2002). It is becoming increasingly important to better understand the fate of N in our arable soils and to increase nitrogen use efficiency.

Due to the pivotal role that AO play in nitrification, it is important that we understand the factors that shape the growth and activities of these communities. Many studies have investigated the abiotic conditions that influence the abundance of AO and their metabolic activities; pH, temperature, soil depth, and fertilization history have been identified as important drivers of AO population sizes (Di et al., 2010; Nicol et al., 2008; Ouyang et al., 2017; Tourna et al., 2008; Wessén et al., 2010). However, there have been relatively few investigations of the biotic factors that may drive AO abundance. For example, the rhizosphere effect, where root exudation and increased rates of nutrient cycling exist near the root, may increase AO abundances (Ai et al., 2013; Dias et al., 2012; Hussain et al., 2011). However, potentially in contrast, nitrification rates have also

been found to be lower along the root surface (Herman et al., 2006). Therefore, biotic interactions may play an important, though unclear, role in determining AO abundance and activity in soils. There is also limited knowledge about biotic interactions near the root between AO and other microorganisms that may affect their growth.

Arbuscular mycorrhizal fungi (AMF) form symbioses with plant roots in which their hyphae extend into soil to acquire nutrients for the plant in exchange for photosynthetic carbon, acting as an extension of the root system. It is becoming evident that AMF play a more significant role in soil N cycling than previously thought (Veresoglou et al., 2012). For instance, AMF can absorb inorganic N from root litter and this nutrient displacement can alter the surrounding microbial community (Nuccio et al., 2013). Increased uptake of inorganic N by AMF inoculated plants can be significant enough to reduce leaching of NH_4^+ and NO_3^- from soils (Asghari and Cavagnaro, 2011; Bender et al., 2014; Cavagnaro et al., 2015; Corkidi et al., 2011; Köhl and van der Heijden, 2016). Because AO are relatively poor competitors for NH_4^+ compared to other heterotrophic microbes (Verhagen and Laanbroek, 1991; Verhagen et al., 1995), it has been hypothesized that AMF and AO may compete for the same NH_4^+ pool, particularly since AMF are thought to prefer NH_4^+ to NO_3^- when transferring N to plant hosts (Govindarajulu et al., 2005; Tanaka and Yano, 2005). Thus, AMF may have an inhibitory effect on AO growth. Various studies have investigated this possible competitive interaction between AMF and AO, but results have been inconclusive. AMF have been found to increase, decrease, or have little impact on AO population sizes or activity (Amora-Lazcano et al., 1998; Cavagnaro et al., 2007; Chen et al., 2013; Veresoglou et al., 2011).

Despite previous efforts, the inconsistency of results between experiments has made it difficult to understand the interactions between AMF and AO, and if there is any agricultural application for AMF in reducing nitrification. In this study, our goal was to investigate the interactions between AMF and AO in contrasting agricultural systems. We hypothesized that AMF would decrease soil AO abundance and that this effect would be larger in a diversified (four-year rotation, manure amendments) compared to a conventional (two-year rotation, inorganic fertilization) managed soil due to lower NH_4^+ availability and increased AMF community function in the diversified system. An AMF deficient (AMF-) genotype of maize (loss of function alleles in the dmi1 gene) was compared to an AMF proficient (AMF+) wild-type progenitor. These corn genotypes were grown in rhizotrons filled with soil from conventional and diversified managed fields. The abundances of AOA and AOB amoA genes (the enzymes responsible for the rate limiting step of nitrification) were measured to determine changes in AO abundance in both the rhizosphere and bulk soils. Our findings indicate that root proximity and soil management, rather than AMF, significantly affected AO population sizes.

Methods

Field Site and Soil Collection

We collected soils from the Iowa State University Marsden Long-Term Cropping System Experiment located in Boone County, IA (42°01’ N; 93°47’ W; 333 m above sea level) (Davis et al., 2012). The soil varies across the experimental site but mostly comprises of Clarion loam (fine-loamy, mixed, superactive, mesic, Typic Hapludolls), Nicollet loam (fine-loamy, mixed, superactive, mesic, Aquic Hapludolls), and Webster silty clay loam (fine-loamy, mixed, superactive, mesic, Typic Endoaqualls) (Davis et al. 2012). The site had been used for conventional corn and soybean agriculture before the

experiment was established in 2002. We chose two treatments within the Marsden site experimental design with which to conduct our experiment: conventional and diversified. The two systems are managed to provide the same levels of N, but in different forms. The conventional system comprises a two-year rotation (soybean, corn) with inorganic N fertilizer and herbicide application comparable to surrounding commercial farms. The diversified system comprises a four-year rotation (soybean, corn, oat/alfalfa, alfalfa) that receives composted manure and reduced inorganic fertilizer side dresses as needed.

For this experiment, we collected soil from a single block of the Marsden experiment on 5/6/16, directly after corn seeding. At planting, the conventional field had been fertilized (UAN-32, 100 lbs/acre) and the diversified field had been fertilized with composted manure the previous fall (11/11/15, 7.6 tons/acre). We collected soil from the first 0-20 cm at roughly evenly-spaced intervals along the entire length of the plot (83.8 m x 18.3 m) between the planted rows. The soil was air-dried in the lab for 2-3 days and then homogenized through surface-sterilized 8 mm sieves. At this time we removed plant debris, worms, and rocks. We stored the homogenized soil in surface sterilized 5-gallon buckets at 15°C until set up of the experiment.

Rhizotrons and Growth Conditions

We used an AMF deficient maize genotype (loss of function alleles in the dmi1 gene) and the AMF proficient progenitor to assess the effects of AMF on soil microbial communities. This approach permitted minimal disturbance of the native microbial seed bank. Furthermore, with this method we could take advantage of the native AMF communities as shaped by soil management rather than inoculating after sterilization to establish a non-AMF control.

We planted surface-sterilized, pre-germinated maize seeds of both genotypes into rhizotrons (7.5 in. x 15.75 in. x 2 in.) filled with either conventional or diversified soil to a bulk density of 1.1 g soil/cm^3. The rhizotrons were a modified version of those used in Jaeger et al. (1999) and consisted of five plastic faces held together by duct tape, with one clear face used to observe root growth that was covered by black felt. Our experimental design was comprised of two factors, soil management type and AMF genotype, and was replicated 5 times per treatment for a total of 20 rhizotrons. At set up, we took subsamples of the conventional and diversified soils and sent them to the Iowa State University Plant and Soil Analysis Laboratory for initial soil nutrient and property measurements using their standard protocols (Eliason et al., 2015). We grew plants under fluorescent growth lamps (6400K T5 Hydrofarm Agrobrite) on a 16 hr light, 8 hr dark cycle at room temperature (20-23°C). Rhizotron water content was adjusted to 60% water holding capacity 3 days per week and we randomized the placement of rhizotrons under the growth lamps at each watering. The maize plants grew for a total of 10 weeks to allow adequate AMF colonization and hyphal growth in the soil.

Harvest Protocol

At harvest, we removed the face of each rhizotron to slide the contents into a large, sterilized autoclave tub for sample collection. Pre-sterilized materials were used for every step and surface-sterilized gloves were used whenever direct handling of sample material was necessary. We cut the plant shoot at the base of the roots and placed the shoots in a drying oven to determine plant dry weight and nutrient contents. Roots were gently extracted from the soil and then shaken three times into a separate tub to remove non-rhizosphere soil. We defined the rhizosphere as the soil aggregates that were left clinging

to the root at this point. The bulk soil was defined as the soil remaining in the original tub that was not removed with the roots.

To collect rhizosphere soil, we first cut the root in half at the crown. We placed one half of the roots into ice-cold DI water and refrigerated it for later AMF root colonization quantification. The other half of the root was placed into a 60 ml Falcon tube with 45 ml of ice-cold phosphate buffer (0.1 M) solution. We vortexed this slurry for 1 min to release soil particles from the root. After removing the root, we then centrifuged the soil slurry at 10,000xG at 4°C to pellet the soil. These steps were repeated two additional times to remove all visible soil particles from the root surface. The three resulting soil pellets were pooled and resuspended in 4 ml ice-cold DI water via vortexing. We pipetted the soil slurry into 2 ml centrifuge tubes, centrifuged at 10,000xG for 1 min, and decanted the supernatant. The resulting rhizosphere soil pellet was frozen immediately at -20°C then later transferred to -80°C. In some cases, root biomass was not sufficient for both rhizosphere soil collection and AMF colonization quantification so instead the entire root system was used for rhizosphere soil collection and AMF root colonization quantification was forgone. This lead to sample sizes of n = 4 for AMF- genotype treatments and n = 5 for AMF+ genotype treatments. Cleaned roots were dried and weighed.

Concurrently, we collected bulk soil samples using the following protocol. The soil was first homogenized for 2 min and up to 5 g was placed into 2 ml centrifuge tubes. We froze these samples immediately at -20°C and later transferred them to a -80°C freezer. Lastly, 100 g of bulk soil from each sample were subsampled into Whirl-Pak bags and stored at -20°C then later at -80°C for soil property measurements.

Bulk soil and plant analyses were conducted by the Iowa State University Soil and Plant Analysis Laboratory using their standard protocols (Eliason et al., 2015; Kalra, 1998). Briefly, shoot N was determined via combustion analysis and shoot P was determined via nitric acid microwave digestion. Soil NH_4^+ and NO_3^- concentrations were determined colorimetrically, phosphorus was measured by Mehlich-3 analysis on an ICP, and pH was also measured with an electrode in a soil and water slurry. Shoot N content was interpreted in terms of total N in the shoot, and was calculated by multiplying the percentage N of the shoot by the total dry weight of the shoot.

AMF Biomass and Root Colonization

Soil AMF biomass abundance was determined using the modified PLFA FAME protocol of Gutknecht et al. (2012). First, we freeze-dried a portion of each rhizosphere or bulk soil sample prior to grinding them using a surface-sterilized mortar and pestle. For each sample, up to 1 g dry-weight soil was used for PLFA extractions. Phospholipids were extracted from the soil three times using a chloroform-methanol-citrate buffer mixture (2:4:1.8 v/v/v), and were then saponified. Strong acid methanolysis (325 ml HCl and 50 ml methanol) was then performed to convert phospholipids into fatty acid methyl esters. Next, we extracted fatty acid methyl esters from the aqueous to the organic phase of the solution using hexane. We used a base wash (300 mM sodium hydroxide solution) to remove impurities. Gas chromatography (GC/MS) was used to identify and quantify fatty acids from each extraction. We used a Zebron ZB-5 (30 m x 0.32mm x 0.1 um) and an Agilenta 7890 gas chromatograph coupled to an Isoprime 100 mass-selective detector (Americas, Mt. Laurel, NJ, USA). The C13:0 fatty acid was used as an internal standard to quantify individual peaks and the 16:1ω5 signal fatty acid was used to measure AMF biomass relative to soil dry weight. This biomarker's use for AMF quantification is

limited, but the controlled nature of this study and the nature of our extraction procedure, where phospho and neutral lipids are combined, allow for a qualified use of this fatty acid (Ngosong et al., 2012). During the protocol, two samples were lost leading to sample sizes of n = 4 for AMF+ and AMF- diversified soil treatments and n = 5 for AMF+ and AMF- conventional soil treatments.

We used a modification of the method developed by Giovenetti and Mosse (1980) to quantify the AMF root colonization rate. Each root subsample was washed with DI water and cut into 2 cm pieces. We patted roots dry using paper towels prior to transferring 0.75 g of each sample into biopsy cassettes for clearing and staining. The clearing and staining procedure was carried out as follows: submerge roots in 5% KOH at 90°C for 2 hrs, rinse six times with DI water, submerge in 1% HCl at room temperature for 1 hr, submerge in trypan blue solution (250 ml lactic acid, 250 ml glycerol, 7.5 ml Thermo Scientific Hyclone Trypan Blue Solution) at 90°C for 1 hr, and rinse three times with DI water. We performed all incubations in a pre-heated water bath. We stored the cleared and stained roots in a glycerol solution (1:1:1 solution of lactic acid, glycerol, DI water) at 4°C until quantification. Leftover root sample was oven-dried, weighed, and added to the weight of the freeze-dried roots to calculate approximate total root dry mass.

Stained root pieces were examined at 40x magnification using a dissecting microscope to visually quantify AMF colonization with the grid-line petri method (Giovannetti and Mosse, 1980). We placed stained root segments on a 0.5” grid-line petri dish and counted intersections as colonized or uncolonized. At least 100 intersections were counted per plate. Each root sample was rearranged and recounted an additional 2 times, generating

three counts for each root sample that were then averaged. Percent root colonization was derived using the following equation: (colonized intersections/total intersections) x 100.

DNA Extraction and qPCR

We used PowerLyzer PowerSoil DNA extraction kits (Mobio, Carlsbad, CA) to extract DNA from soil samples using their standard protocol, including bead-beating soils with a PowerLyzer (Mobio, Carlsbad, CA) at 2500 rpm for 45 seconds and eluting DNA in 50 ul of solution C6. We stored all DNA samples at -80°C.

Quantitative PCR was used to measure AOA and AOB amoA gene abundance. The AOB amoA gene primers were amoA-1F and amoA-2R and the AOA amoA gene primers were Arch-amoAF and Arch amoAR (Francis et al., 2005; Rotthauwe et al., 1997). We used a 1:1:1 mixture of known concentrations of 3 synthesized amoA gene DNA (G-blocks, Invitrogen) fragments the same length as our target amplicons, each with 0-2 mismatches with the primers. The AOB amoA gBlocks were derived from N. Europea, N. briensis, and a terrestrial uncultured clone (GenBank accessions: KU747122.1, U76553.1, EF207201.1). The AOA amoA gBlocks were derived from candidatus Nitrosphaera sp. and two terrestrial uncultured archeon clones (GenBank accessions: FR773159.1, KP984498.1, KF004126.1). We performed all reactions in 20 µl volumes containing 10 ng of template DNA, 0.3 µM of each primer, 0.005 mg/µl of BSA (for AOB amoA reactions only), 10 ul of PerfeCTa SYBR Green Fastmix Reaction mix (QuantaBio, Beverly, MA), and the remaining volume nuclease-free water. Thermocycler conditions for both AOA and AOB amoA gene reactions were as follows: an initial denaturing step of 95°C for 3 min, and 40 cycles of 95°C for 30 sec, 55°C for 1 min, and 72°C for 45 sec. All reactions were performed on a Realplex2 Mastercycler (Eppendorf, Hauppauge, NY). Each reaction with sample and standard template was performed in

triplicate, along with three negative no-template controls. Standard curves were generated with linear dilutions of the AOB or AOA gBlock mixtures. Product specificity was confirmed by melting curve analysis and select samples were verified via gel electrophoresis.

Statistical Analyses

All statistical analyses were performed in R version 3.3. For AMF biomass, root colonization, and amoA gene abundance data, main effects and interactions of AMF status, soil management, and root proximity were determined via multi-way ANOVA. The main-effects and interactions of AMF status and soil management on soil property data were determined via two-way ANOVA. For soil P concentrations at the end of the experiment, Kruskal-Wallis tests were instead applied to explore main effects due to high heterogeneity of variability of the data. Post-hoc tests were determined via Tukey's HSD when all comparisons were of interest or with pair-wise T-tests with a Bonferroni adjustment when only specific comparisons were of interest. Statistical comparisons between before and after-experiment soil property data were determined via Student's or Welch's T-tests as deemed appropriate after examining the data's normality and heterogeneity of variability. Log transformations were applied to the data as necessary to improve normality and heterogeneity of variance. For PLFA data, an inverse hyberbolic sin transformation was applied. Normality and heterogeneity of the data were evaluated using Shapiro-Wilk and Levene's tests respectively as well as visual examination.

Results

Mycorrhizal Abundance, Colonization, and Plant Nutrition

The AMF 16:1ω5 fatty acid marker abundance was significantly reduced in the AMF- treatments compared to the AMF+ treatments ($P < 0.0001$). However, soil management and rhizosphere effect, along with all interactions, had no significant impacts (Figure 3.1). Likewise, AMF root colonization was significantly greater in AMF+ plant roots compared to AMF- plant roots ($P < 0.0001$) but did not differ significantly between soil management types (Table 3.1).

Plant genotype significantly affected plant growth (Table 3.1). AMF+ plant shoots had significantly greater dry-weight ($P < 0.0001$) compared to AMF- plant shoots. Soil management significantly influenced shoot biomass, with greater biomass in AMF+ plants grown in conventional soil compared to AMF+ plants grown in diversified soil ($P < 0.05$). Root dry-weight followed similar trends; the AMF+ plants had significantly greater root biomass than the AMF- plants ($P < 0.001$ both), although no differences were found between plants grown in soil from different management regimes.

There were significant effects of plant genotype and soil management on shoot N content ($P < 0.001$ and $P < 0.05$ respectively) (Table 3.1). Shoot total N was greater in AMF+ plants compared to AMF- plants grown in conventional soil ($P < 0.001$). However, AMF+ and AMF- plants grown in the diversified soil did not significantly differ in shoot total N content. Additionally, AMF+ plants grown in conventional soil had significantly greater shoot total N compared to AMF+ plants grown in diversified soil. Shoot P concentration followed different trends (Table 3.1). There was a significant effect of genotype but not soil management on shoot P concentration ($P < 0.001$ and $P < 0.05$ respectively). Shoot P concentration was significantly higher in the AMF+ plants

compared to the AMF- plants grown in conventional soil ($P < 0.01$), but the same was not true for plants grown in diversified soil. Plants grown in differing soil management types did not have significantly different shoot P concentrations regardless of plant genotype.

Soil Properties Before and After

Immediately before planting the rhizotrons the conventional and diversified soils had equivalent total N, NO_3^-, P, and pH levels ($P > 0.05$) (Table 3.2, Figure 3.2). Soil NH_4^+ was the only variable with significantly higher concentrations in the conventional soil compared to the diversified soil ($P < 0.05$). After the experiment was completed soil NH_4^+, NO_3^-, P, and pH showed different responses to AMF treatment and soil management (Table 3.2, Figure 3.2). Soil NH_4^+ was significantly, though only slightly, greater in AMF+ and AMF- planted treatments independent of soil management ($P < 0.01$ both). The opposite was true for soil NO_3^- concentration, which was significantly decreased in the AMF+ planted soils compared to the AMF- planted soils for both soil management types ($P < 0.0001$ both). Additionally, the AMF- planted treatment contained higher NO_3^- concentrations than the AMF- planted treatment in conventional compared to diversified soils ($P < 0.05$). Soil P showed a significant main effect of AMF status ($P < 0.05$) and was greater in the AMF- planted soil compared to the AMF+ planted soil regardless of soil management. Soil pH was significantly higher in the AMF+ planted conventional soil compared to all other treatments ($P < 0.05$). Total N in the soil did not differ significantly due to any treatments.

We also compared “before” and “after” soil property data (Table 3.2, Figure 3.2) in order to determine how soil properties changed over the course of the experiment. Inorganic NH_4^+ concentrations were much lower at the end compared to the beginning of the experiment in all treatments ($P < 0.01$). Soil inorganic NO_3^- concentrations were

much greater at the end of the experiment compared to the beginning in the AMF- planted soils ($P < 0.001$ both) but did not change significantly from beginning to the end of the experiment in the AMF+ planted soils. Soil total N did not change significantly from the start to the end of the experiment except for in the case of the AMF- conventional soil, where it appears to have increased slightly ($P < 0.05$). Soil P significantly increased at the end of the experiment compared to the beginning in the AMF- conventional soil, but no other treatments experienced a significant shift in P concentration over the course of the experiment. Soil pH did not change over the course of the experiment in either soil systems or in response to AMF.

Archaeal and Bacterial amoA Gene Abundances

We observed no changes in AOB or AOA amoA gene abundance in response to AMF treatment, but instead observed differences based on soil management type or root proximity (Figure 3.3). AOA amoA gene abundances were significantly greater within rhizosphere soil compared to bulk soil regardless of AMF or soil treatment ($P < 0.0001$). Specifically, AOA amoA abundance was significantly greater in the rhizosphere compared to the bulk soil of all but the AMF+ conventional soil treatment. AOB amoA abundance, alternatively, was significantly greater in the conventional soil compared to the diversified soil regardless of AMF treatment or root proximity independent of plant AMF status ($P < 0.05$ both).

Discussion

Due to ecological damage caused by nitrate leaching in agricultural soils, understanding the ecology of AO and what variables control their growth and metabolism has become increasingly relevant. In the current study, we used AMF proficient and deficient maize genotypes to investigate biotic interactions between AMF and AO in

agricultural soils. Contrary to our hypothesis, AMF had no impact on AOA or AOB population abundances in either soil management type. Rather, AOA and AOB population sizes responded to proximity to the root or management history respectively. Our data do not support our hypothesis that AMF decreased NH_4^+ availability to AO in agricultural soils. Instead, NH_4^+ concentration in the AMF+ planted soils slightly increased. This finding supports a previous observation that AMF can enhance ammonification to meet plant demand (Atul-Nayyar et al., 2009).

In the AMF- planted soils, inorganic NO_3^- concentration increased over the course of the 10-week experiment, indicating that nitrification occurred. In contrast, in the soils planted with the AMF+ genotype, the NO_3^- concentrations remained low over the course of the experiment. Although pool size measurements cannot capture the full dynamics of inorganic N cycling in soils, the evidence at hand suggests two possible explanations for this finding: 1) AMF+ plants suppressed nitrification or 2) AMF+ plants had a superior ability to capture soil NO_3^- compared to AMF- plants. The second scenario is more likely given that neither AOA nor AOB population sizes, which have been closely correlated to nitrification rates in soils (Wessén and Hallin, 2011), were altered due to AMF. It is likely that AMF increased NO_3^- removal from the soil either by direct uptake through hyphae or indirectly by increasing plant root biomass and hence absorptive capacity. AMF greatly increased plant shoot and root biomass in this experiment, indicating an indirect mechanism of inorganic NO_3^- absorption. Of course, possible pleiotropic effects of knocking out the dmi1 gene that may have contributed to the differences in root biomass must also be noted, though the effects of this gene on plant growth have not been thoroughly investigated. It is also possible that AMF decreased NO_3^- pool sizes via direct

absorption by hyphae. Though it has been shown in controlled conditions that AMF prefer to transfer NH_4^+ to the plant (Govindarajulu et al., 2005; Tanaka and Yano, 2005), it has also been reported that AMF can reduce the concentration of NO_3^- in soils disproportional to the correlated increase in plant root biomass, indicating direct uptake by fungal hyphae (Asghari and Cavagnaro, 2011).

Taken together, these data indicate that AMF in agricultural soils may have a greater impact on soil NO_3^- rather than NH_4^+ availability. In an agricultural soil, where rapid nitrification can occur, AMF and maize alike may shift preference from more easily metabolized NH_4^+ to NO_3^-, which is known as a preferred inorganic N source for higher plants when NH_4^+ is not readily available (Haynes, 1986; Taylor and Bloom, 1998). Because AO rely on inorganic NH_4^+ to fuel their metabolism, but AMF appear to have directly or indirectly reduced NO_3^- rather than NH_4^+ availability, AO population sizes were unaffected (Figure 3.4). Additionally, NO_3^- removal, rather than nitrification inhibition, may be an explanation for decreased rates of NO_3^- leaching from AMF colonized soils observed in various studies (Asghari and Cavagnaro, 2011; Cavagnaro et al., 2011; Corkidi et al., 2011; Köhl and van der Heijden, 2016).

These findings support those of Cavagnaro et al. (2007) who used a similar tomato AMF deficient/proficient experimental design in a field study of an organically managed agricultural soil and found no impact of AMF on AOB population sizes. However, other experiments that investigated AMF and AO interactions in non-agricultural soils have yielded differing results, either an increase or decrease in AO abundance or activity (Amora-Lazcano et al., 1998; Chen et al., 2013; Veresoglou et al., 2011). In light of evidence from these other studies, this suggests that the N-rich nature of agriculturally

managed soils decreases the influence of AMF on NH_4^+ availability and thus ability to impact AO population sizes, possibly by shifting preference toward NO_3^- absorption under conditions that favor nitrification. N fertilization can also decrease AMF community diversity and growth, which could also decrease the impact of AMF on NH_4^+ absorption (Bradley et al., 2006; Brito et al., 2012; Oehl et al., 2003). Future studies should develop a more mechanistic understanding of how AMF influence soil N availability across many soil types, various agricultural fertilization regimes, and within various specific AMF genera or species to see how these factors interact.

Rather than responding to the presence or absence of AMF, AOA abundances were instead altered by root proximity while AOB population sizes were influenced by soil management. The AOA population size was greater in the rhizosphere soil compared to the bulk soil, independent of AMF status or soil management. It has been documented that AOA are not solely reliant on nitrification as an energy source and may be capable of mixotophic growth on organic substrates (Jia and Conrad, 2009; Prosser and Nicol, 2008; Wessén et al., 2010). Several studies have reported increased AOA population sizes within the plant rhizosphere, but mechanisms for this trend remain unknown (Ai et al., 2013; Chen et al., 2008; Herrmann et al., 2008; Hussain et al., 2011). Based on these findings, it is possible that AOA populations increased within the maize rhizosphere by growing mixotrophically on root exudates, rather than based on the availability of NH_4^+.

In contrast, the AOB population size was greater in the conventional soil than in the diversified soil irrespective of plant AMF status or proximity to the root. This result is consistent with many studies that report AOB population sizes increase when soils are inorganically fertilized (Giguere et al., 2015; Ouyang et al., 2016; Sterngren et al., 2015;

Ying et al., 2017). The impact of AMF on the soil environment was not sufficient to overcome the larger influence of fertilization on AOB population sizes. Although NH_4^+ availability decreased over the course of the experiment and was similar in both soils by the end, it is likely that legacy effects maintained a higher AOB population in the conventional soil due to the higher NH_4^+ availability at the beginning of the experiment. AO are known to be slow growers and thus population sizes can take much longer to react to environmental changes compared to other prokaryotes (Sharma and Ahlert, 1977). Unlike AOA, proximity to the root did not influence AOB population sizes. However, the effect of the rhizosphere on AOB abundance is unclear; in many studies AOB population sizes have been reported to increase in the rhizosphere compared to bulk soil (Ai et al., 2013; Dias et al., 2012; Glaser et al., 2010; Hussain et al., 2011), while in others AOB abundances are unchanged (Chen et al., 2008; Herman et al., 2006; Rudisill et al., 2016), as was observed in this study. More research is necessary to understand the impact of roots on AO population sizes and activities.

Overall, these results indicate differing ecological niches for AOB and AOA within agricultural soils. Heterotrophic growth may be a driving force of AOA growth in agricultural systems rather than fertilization, while inorganic fertilization is a greater driver of AOB population sizes instead. This interpretation is supported by many studies that find ammonium application has large effects on AOB but not AOA population sizes and activities (Ai et al., 2013; Di et al., 2010; Glaser et al., 2010; Jia and Conrad, 2009; Ouyang et al., 2017, 2016; Sterngren et al., 2015; Wu et al., 2011; B. Zhang et al., 2012).

Conclusions

In conclusion, in two contrasting agricultural systems, AMF growth in soil microcosms did not affect AO population sizes. Rather, the rhizosphere, possibly via growth on root exudates, increased AOA abundance irrespective of soil management, and an inorganically fertilized management system increased AOB population sizes. Rather than reduce NH_4^+ availability in the soil as hypothesized, AMF slightly increased NH_4^+ pool sizes and greatly decreased NO_3^- concentration in the soil. We propose that, in N-rich soils where nitrification can occur rapidly, AMF may instead aid the plant in increased NO_3^- uptake via greater root biomass and that the influence of AMF on AO population sizes in agricultural soils is therefore limited. Other factors, such as the rhizosphere effect and fertilization instead have a greater impact on AO in agricultural soils. Future work investigating interactions between AMF and AO should aim to compare soils managed with differing levels of N fertilization. This would allow us to further delineate the conditions under which AMF may alter AO abundance and activities, and identify targets for agricultural or land-management practices that make the best use of the ecosystem services AMF can provide.

Table 3.1. AMF colonization and plant properties. AMF- refers to the mycorrhizae-deficient mutant genotype and AMF+ refers to the mycorrhizae proficient genotype. Numbers represent treatment means and letters differentiate significance at $P < 0.05$ as determined by Tukey's HSD for plant properties or two-way ANOVA for AMF colonization.

Treatment		AMF Colonization	Dry Weight		Shoot Nutrients	
Mycorrhization	**Soil Management**	**(%)**	**Shoot (g)**	**Root (g)**	**Total N (g)**	**P (mg kg^{-1})**
AMF-	Conventional	28.25^{a}	2.14^{a}	0.30^{a}	0.049^{a}	1190^{a}
AMF-	Diversified	22.66^{a}	3.00^{a}	0.37^{a}	0.052^{a}	719^{b}
AMF+	Conventional	52.83^{b}	15.66^{b}	1.35^{b}	0.170^{b}	1626^{a}
AMF+	Diversified	51.79^{b}	9.34^{c}	0.89^{b}	0.105^{a}	1662^{a}

Table 3.2. Soil properties before and after the experiment. AMF- refers to the mycorrhizae-deficient mutant genotype and AMF+ refers to the mycorrhizae proficient genotype. Numbers represent treatment means and letters differentiate significance within Before/After categories at $P < 0.05$ as determined by Tukey's HSD. Asterisks indicate significance between before and after values within the same soil management type at $P < 0.05$ according to appropriate T-tests. † Kruskal-Wallis tests were instead used to test main effects due to high heterogeneity of variability.

	Treatment						
	Mycorrhization	**Soil Management**	**Total N (%)**	**Ammonium (mg/kg)**	**Nitrate (mg/kg)**	**Phosphorus (mg/kg)†**	**pH**
Before	N/A	Conventional	0.23^{a}	35.67^{a}	1.53^{a}	21.67^{a}	6.58^{a}
	N/A	Diversified	0.23^{a}	18.00^{b}	1.53^{a}	25.67^{a}	6.49^{a}
After	AMF-	Conventional	0.25^{a*}	2.44^{a***}	37.24^{a***}	27.20^{a*}	6.59^{a}
	AMF-	Diversified	0.23^{a}	2.34^{a***}	17.92^{b***}	36.20^{a}	6.43^{a}
	AMF+	Conventional	0.24^{a}	3.30^{b***}	1.56^{c}	22.40^{b}	6.91^{b}
	AMF+	Diversified	0.24^{a}	3.18^{b***}	1.20^{c}	34.00^{b}	6.59^{a}

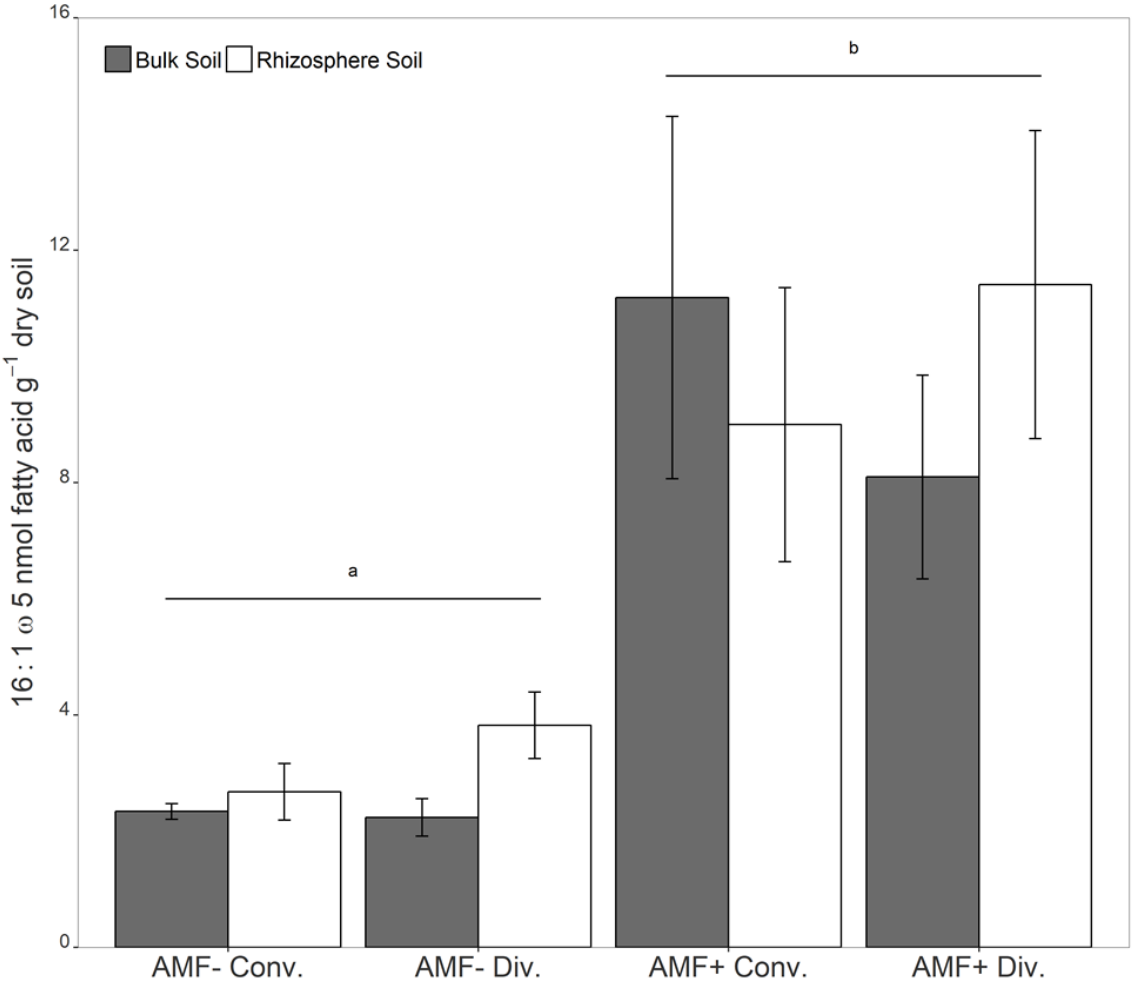

Figure 3.1. AMF biomass as determined by PLFA FAME. AMF- refers to the mycorrhizae-deficient mutant genotype and AMF+ refers to the mycorrhizae proficient genotype. Conv. and Div. refer to conventional and diversified managed soils. Bars with different letters indicate significant difference at $P < 0.05$ of pooled treatments as determined by multi-way ANOVA on inverse-hyperbolic sin transformed data.

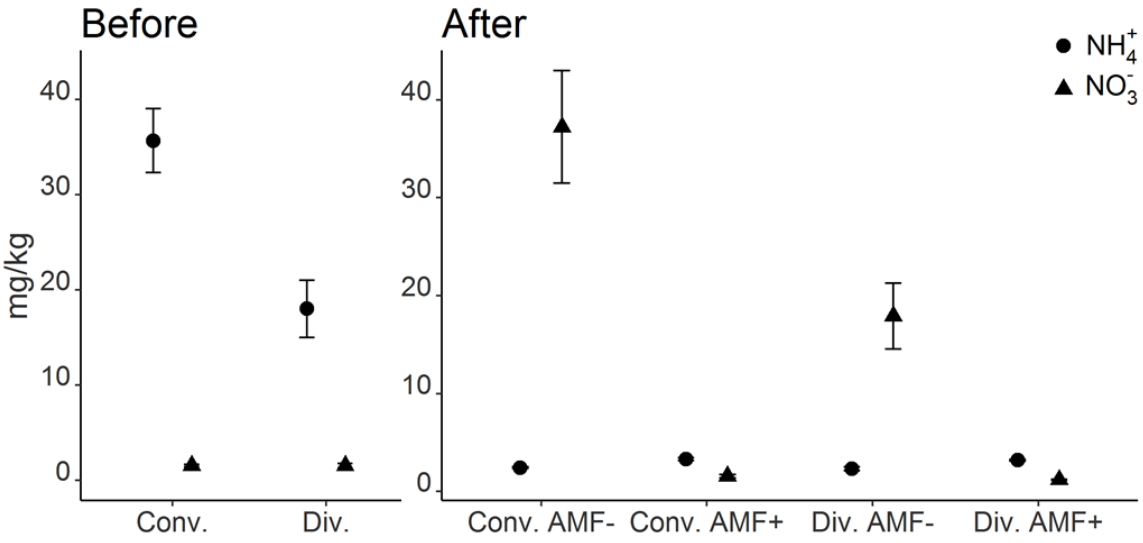

Figure 3.2. Side-by-side comparison of bulk soil ammonium and nitrate concentrations immediately before set up of the experiment and immediately after harvest.

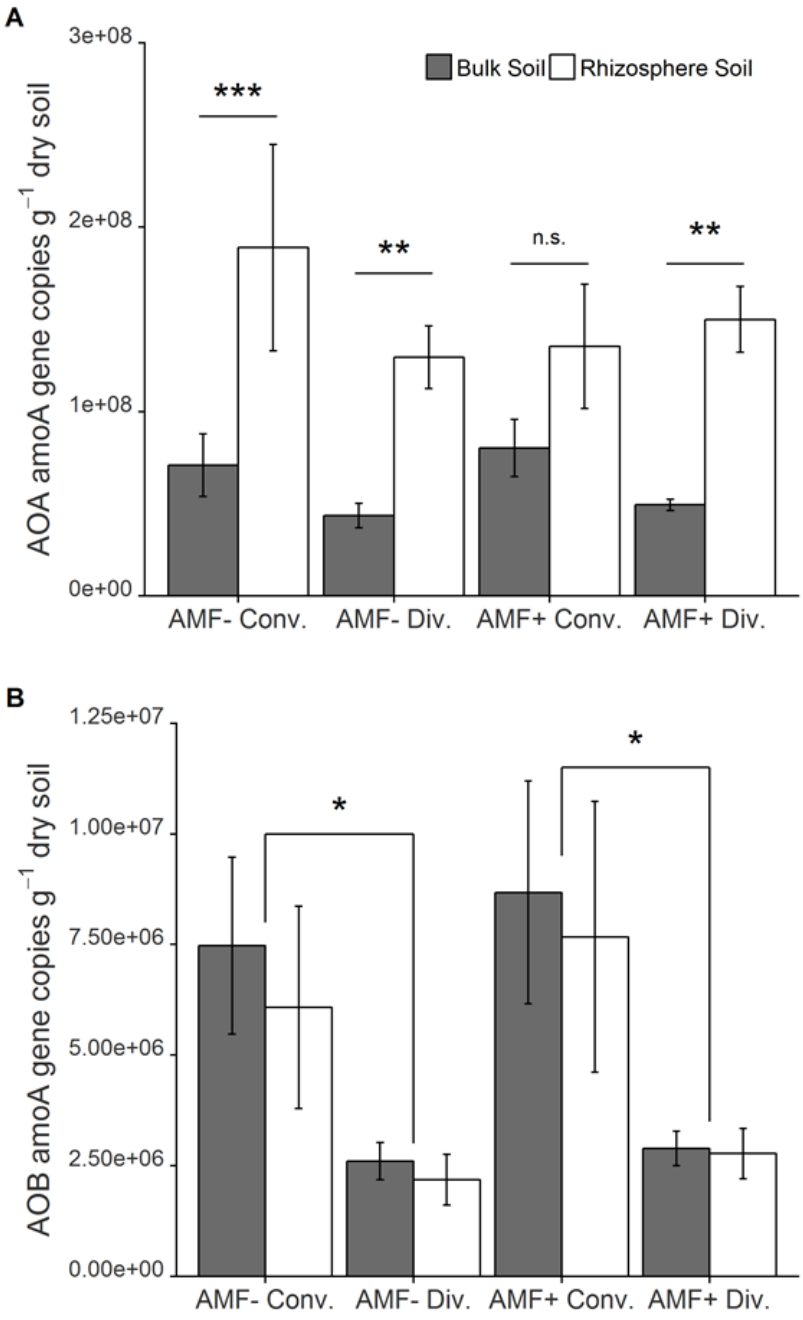

Figure 3.3. Ammonia oxidizer abundance in bulk and rhizosphere soils as determined by qPCR of the amoA gene. **A)** AOA amoA gene abundance and **B)** AOB amoA gene abundance. AMF- and AMF+ refer to rhizotrons planted with the AMF deficient and AMF proficient maize genotypes respectively. Conv. and Div. refer to conventional and diversified soils. Lines over bars in plot A indicate significance between rhizosphere and bulk soil of that treatment. In plot B, lines with a star connecting groups of treatments denote significance between bulk and rhizosphere soil. Significance was determined via paired T-tests with Bonferroni P-adjustment.

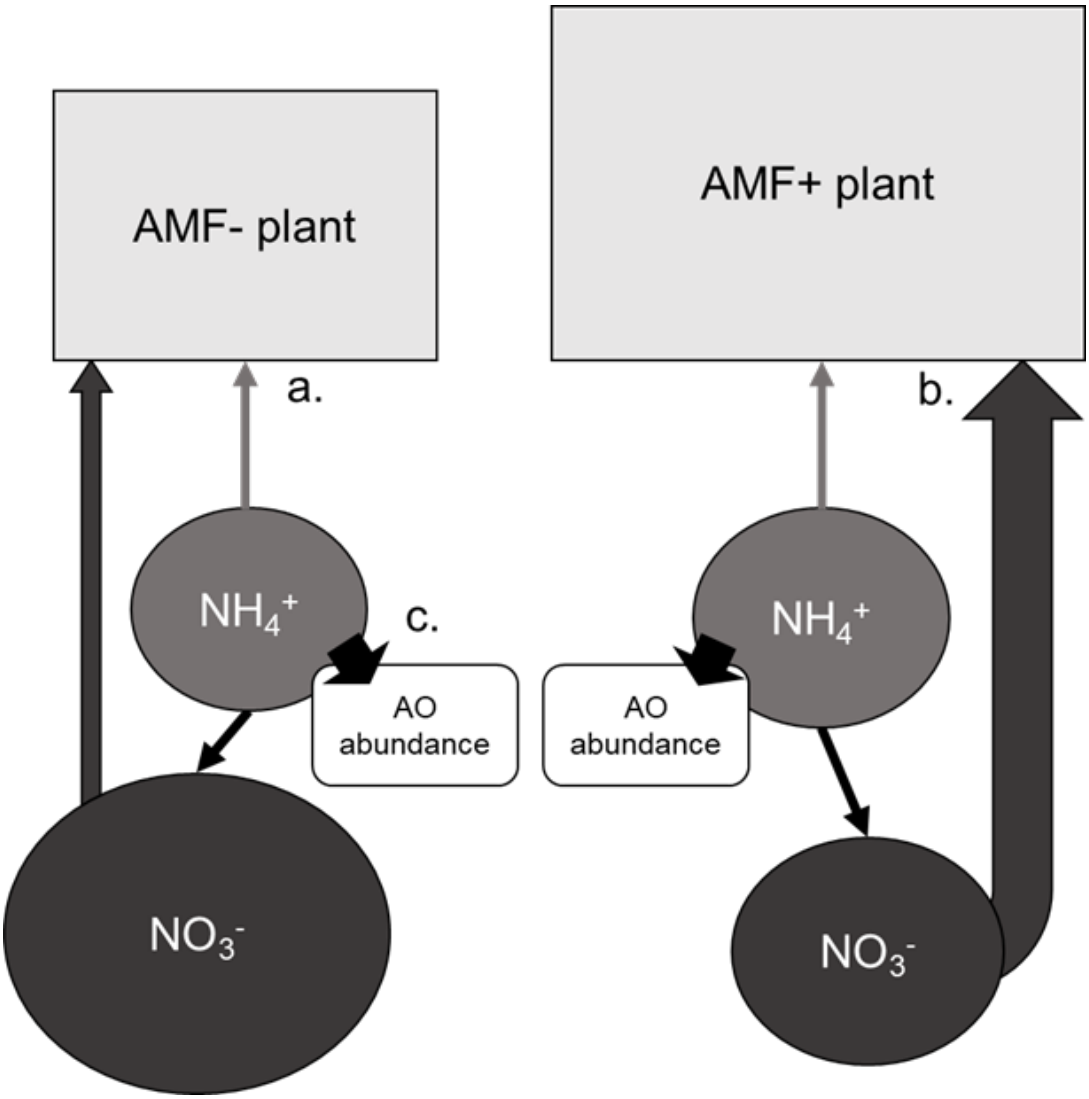

Figure 3.4. Theoretical model of AMF interactions with AO in N rich soils. AMF- and AMF+ refer to AMF deficient and proficient maize genotypes respectively. A) Similar uptake of ammonium from soil by AMF- and AMF+ plants, B) Increased uptake of nitrate by AMF+ plants due to either increased root biomass or direct absorption by AMF hyphae, C) Similar availability of ammonium to AO in soils planted with AMF- and AMF+ genotypes, and thus no significant differences in AO population sizes.

CHAPTER 4. SUMMARY AND CONCLUSIONS

Sustainable food production is a matter of increasing importance, and improving nitrogen use efficiency is a key component of agricultural sustainability. Due to their roles in nutrient mineralization and plant-growth promotion, soil microbes are a potential mechanism for achieving better nitrogen use efficiency. The overall goal of this thesis was to develop a better understanding of root-associated microbes and their potential role in N retention in agricultural soils. The second chapter explored the interaction between maize roots, agricultural management, and root-associated microbial communities to gain a better understanding of the temporal and spatial dynamics of the root-associated microbial communities, and its implications for N cycling in soils. Our results showed that root-associated prokaryotic communities diverge most between cropping systems at a period of high maize N demand, and that there are higher abundances of complex organic matter decomposing families in the diversified system, possibly indicating that root-microbe coupling to degrade manure inputs in this system is a mechanism by which N is better retained. On the other hand, fungal root-associated communities between cropping systems were most different at the first time point and converged in similarity thereafter, indicating that the fungal seedbank as shaped by agricultural management determined the first-colonizers of the root, and then the different communities converged as the plants developed. Furthermore, these trends were mostly detectable in the rhizoplane communities, indicating that root-microbe interactions occur at a much more intimate scale than previously considered.

In the third chapter, the effects of arbuscular mycorrhizae on the population sizes of ammonia-oxidizing bacteria and archaea were investigated in the conventional and

diversified managed soil from the Marsden site. Overall, arbuscular mycorrhizal fungi had no effect on ammonia oxidizer population sizes. However, ammonia-oxidizing archaea were more abundant within the rhizosphere, while ammonia-oxidizing bacteria were more abundant in the conventional compared to the diversified cropping system soil. Rather than decrease the pool size of ammonium in the soil, which ammonia oxidizers use for their metabolism, arbuscular mycorrhizae instead indirectly or directly decreased the pool size of nitrate. This suggests that in N-rich soils, such as these tested, arbuscular mycorrhizae have a greater impact on nitrate pool sizes compared to ammonium pool sizes. This would mean the competitive interaction between ammonia oxidizers and arbuscular mycorrhizae is limited. Overall, both of these studies provide better insight into the complex interactions between soil, plants, and microbes that affect soil N cycling, and how their combined functions can be incorporated into agricultural management for a more sustainable future.

REFERENCES

Alexander, R.B., Smith, R.A., Schwarz, G.E., Boyer, E.W., Nolan, J. V., Brakebill, J.W., 2008. Differences in Phosphorus and Nitrogen Delivery to The Gulf of Mexico from the Mississippi River Basin. Environ. Sci. Technol. 42, 822–830. doi:10.1021/es0716103

Adesemoye, A.O., Kloepper, J.W., 2009. Plant-microbes interactions in enhanced fertilizer-use efficiency. Appl. Microbiol. Biotechnol. 85, 1–12. doi:10.1007/s00253-009-2196-0

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., 2013. Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. Soil Biol. Biochem. 57, 30–42. doi:10.1016/j.soilbio.2012.08.003

Alexander, R.B., Smith, R. a, Gregory, E., Boyer, E.W., Nolan, J. V, Brakebill, W., 2008. Differences in Phosphorus and Nitrogen Delivery to the Gulf of Mexico from the Mississippi River Basin Related links : 42, 11–12.

Amora-Lazcano, E., Vázquez, M.M., Azcón, R., 1998. Response of nitrogen-transforming microorganisms to arbuscular mycorrhizal fungi. Biol. Fertil. Soils 27, 65–70. doi:10.1007/s003740050401

Anders, S., Huber, W., 2010. Differential expression analysis for sequence count data. Genome Biol. 11, R106. doi:10.1186/gb-2010-11-10-r106

Asghari, H.R., Cavagnaro, T.R., 2011. Arbuscular mycorrhizas enhance plant interception of leached nutrients. Funct. Plant Biol. 38, 219–26. doi:10.1071/FP10180

Atul-Nayyar, A., Hamel, C., Hanson, K., Germida, J., 2009. The arbuscular mycorrhizal symbiosis links N mineralization to plant demand. Mycorrhiza 19, 239–246. doi:10.1007/s00572-008-0215-0

Baudoin, E., Benizri, E., Guckert, A., 2002. Impact of growth stage on the bacterial community structure along maize roots, as determined by metabolic and genetic fingerprinting. Appl. Soil Ecol. 19, 135–145. doi:10.1016/S0929-1393(01)00185-8

Bender, S.F., Conen, F., Van der Heijden, M.G.A., 2014. Mycorrhizal effects on nutrient cycling, nutrient leaching and N2O production in experimental grassland. Soil Biol. Biochem. 80, 283–292. doi:10.1016/j.soilbio.2014.10.016

Bender, S.F., van der Heijden, M.G.A., 2015. Soil biota enhance agricultural sustainability by improving crop yield, nutrient uptake and reducing nitrogen leaching losses. J. Appl. Ecol. 52, 228–239. doi:10.1111/1365-2664.12351

Bender, S.F., Wagg, C., van der Heijden, M.G.A., 2016. An Underground Revolution: Biodiversity and Soil Ecological Engineering for Agricultural Sustainability. Trends Ecol. Evol. 31, 440–452. doi:10.1016/j.tree.2016.02.016

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486. doi:10.1016/j.tplants.2012.04.001

Berendsen, R.L., Pieterse, C.M.J., Bakker, P.A.H.M., 2012. The rhizosphere microbiome and plant health. Trends Plant Sci. 17, 478–486. doi:10.1016/j.tplants.2012.04.001

Bradley, K., Drijber, R.A., Knops, J., 2006. Increased N availability in grassland soils modifies their microbial communities and decreases the abundance of arbuscular mycorrhizal fungi. Soil Biol. Biochem. 38, 1583–1595. doi:10.1016/j.soilbio.2005.11.011

Brito, I., Goss, M.J., de Carvalho, M., Chatagnier, O., van Tuinen, D., 2012. Impact of tillage system on arbuscular mycorrhiza fungal communities in the soil under Mediterranean conditions. Soil Tillage Res. 121, 63–67. doi:10.1016/j.still.2012.01.012

Broeckling, C.D., Broz, A.K., Bergelson, J., Manter, D.K., Vivanco, J.M., 2008. Root exudates regulate soil fungal community composition and diversity. Appl. Environ. Microbiol. 74, 738–44. doi:10.1128/AEM.02188-07

Burkart, M.R., James, D.E., 1999. Agricultural-Nitrogen Contributions to Hypoxia in the Gulf of Mexico. J. Environ. Qual. 28, 850–859. doi:10.2134/jeq1999.00472425002800030016x

Bushnell, B., 2014. BBMap: A Fast, Accurate, Splice-Aware Aligner [WWW Document]. URL https://sourceforge.net/projects/bbmap/ (accessed 8.29.17).

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K., Madden, T.L., 2009. BLAST+: architecture and applications. BMC Bioinformatics 10, 421. doi:10.1186/1471-2105-10-421

Campbell, B.J., Polson, S.W., Hanson, T.E., Mack, M.C., Schuur, E.A.G., 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. Environ. Microbiol. 12, 1842–1854. doi:10.1111/j.1462-2920.2010.02189.x

Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith, V.H., 1998. Nonpoint Pollution of Surface Waters with Phosphorus and Nitrogen. Ecol. Appl. 8, 559–568. doi:10.1890/1051-0761(1998)008[0559:NPOSWW]2.0.CO;2

Cassman, K.G., Dobermann, A., Walters, D.T., 2002. Agroecosystems, Nitrogen-use Efficiency, and Nitrogen Management. AMBIO A J. Hum. Environ. 31, 132–140. doi:10.1579/0044-7447-31.2.132

Cavagnaro, T.R., Barrios-Masias, F.H., Jackson, L.E., 2011. Arbuscular mycorrhizas and their role in plant growth, nitrogen interception and soil gas efflux in an organic production system. Plant Soil 353, 181–194. doi:10.1007/s11104-011-1021-6

Cavagnaro, T.R., Bender, S.F., Asghari, H.R., Heijden, M.G.A. van der, 2015. The role of arbuscular mycorrhizas in reducing soil nutrient loss. Trends Plant Sci. 20, 283–90. doi:10.1016/j.tplants.2015.03.004

Cavagnaro, T.R., Jackson, L.E., Scow, K.M., Hristova, K.R., 2007. Effects of arbuscular mycorrhizas on ammonia oxidizing bacteria in an organic farm soil. Microb. Ecol. 54, 618–26. doi:10.1007/s00248-007-9212-7

Cesarano, G., De Filippis, F., La Storia, A., Scala, F., Bonanomi, G., 2017. Organic amendment type and application frequency affect crop yields, soil fertility and microbiome composition. Appl. Soil Ecol. 120, 254–264. doi:10.1016/j.apsoil.2017.08.017

Chaparro, J.M., Badri, D. V, Vivanco, J.M., 2014. Rhizosphere microbiome assemblage is affected by plant development. ISME J. 8, 790–803. doi:10.1038/ismej.2013.196

Chaparro, J.M., Badri, D. V., Bakker, M.G., Sugiyama, A., Manter, D.K., Vivanco, J.M., 2013. Root Exudation of Phytochemicals in Arabidopsis Follows Specific Patterns That Are Developmentally Programmed and Correlate with Soil Microbial Functions. PLoS One 8, e55731. doi:10.1371/journal.pone.0055731

Chen, X.-P., Zhu, Y.-G., Xia, Y., Shen, J.-P., He, J.-Z., 2008. Ammonia-oxidizing archaea: important players in paddy rhizosphere soil? Environ. Microbiol. 10, 1978–1987. doi:10.1111/j.1462-2920.2008.01613.x

Chen, Y.-L., Chen, B.-D., Hu, Y.-J., Li, T., Zhang, X., Hao, Z.-P., Wang, Y.-S., 2013. Direct and indirect influence of arbuscular mycorrhizal fungi on abundance and community structure of ammonia oxidizing bacteria and archaea in soil microcosms. Pedobiologia (Jena). 56, 205–212. doi:10.1016/j.pedobi.2013.07.003

Chiarini, L., Bevivino, A., Dalmastri, C., Nacamulli, C., Tabacchioni, S., 1998. Influence of plant development, cultivar and soil type on microbial colonization of maize roots. Appl. Soil Ecol. 8, 11–18. doi:10.1016/S0929-1393(97)00071-1

Corkidi, L., Merhaut, D.J., Allen, E.B., Downer, J., Bohn, J., Evans, M., 2011. Effects of Mycorrhizal Colonization on Nitrogen and Phosphorus Leaching from Nursery Containers. HortScience 46, 1472–1479.

Davis, A.S., Hill, J.D., Chase, C.A., Johanns, A.M., Liebman, M., 2012. Increasing Cropping System Diversity Balances Productivity, Profitability and Environmental Health. PLoS One 7, e47149. doi:10.1371/journal.pone.0047149

DeAngelis, K.M., Brodie, E.L., DeSantis, T.Z., Andersen, G.L., Lindow, S.E., Firestone, M.K., 2009. Selective progressive response of soil microbial community to wild oat roots. ISME J. 3, 168–178. doi:10.1038/ismej.2008.103

Di, H.J., Cameron, K.C., 2002. Nitrate leaching in temperate agroecosystems: sources, factors and mitigating strategies. Nutr. Cycl. Agroecosystems 46, 237–256. doi:10.1023/A:1021471531188

Di, H.J., Cameron, K.C., Shen, J.-P., Winefield, C.S., O'Callaghan, M., Bowatte, S., He, J.-Z., 2010. Ammonia-oxidizing bacteria and archaea grow under contrasting soil nitrogen conditions. FEMS Microbiol. Ecol. 72, 386–94. doi:10.1111/j.1574-6941.2010.00861.x

Dias, A.C.F., Hoogwout, E.F., Pereira e Silva, M. de C., Salles, J.F., van Overbeek, L.S., van Elsas, J.D., 2012. Potato cultivar type affects the structure of ammonia oxidizer communities in field soil under potato beyond the rhizosphere. Soil Biol. Biochem. 50, 85–95. doi:10.1016/j.soilbio.2012.03.006

Drinkwater, L.E., Snapp, S.S., 2007. Nutrients in Agroecosystems: Rethinking the Management Paradigm, in: Advances in Agronomy. pp. 163–186. doi:10.1016/S0065-2113(04)92003-2

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. doi:10.1093/bioinformatics/btq461

Edgar, R.C., 2013. UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nat. Methods 10, 996–8. doi:10.1038/nmeth.2604

Eliason, R., Goos, R.J., Hoskins, B., 2015. Recommended Chemical Soil Test Procedures for the North Central Region, Missouri Agricultural Experiment Station SB 1001.

Eliason, R., Goos, R.J., Hoskins, B., 2015. Recommended Chemical Soil Test Procedures for the North Central Region, Missouri Agricultural Experiment Station SB 1001.

FAO, 2009. Livestock in the balance, The State of Food and Agriculture. Food and Agriculture Organization of the United Nations, Rome, Italy. doi:10.1016/S0140-6736(75)92740-3

Fernandez, A.L., Sheaffer, C.C., Wyse, D.L., Staley, C., Gould, T.J., Sadowsky, M.J., 2016. Structure of bacterial communities in soil following cover crop and organic fertilizer incorporation. Appl. Microbiol. Biotechnol. 100, 9331–9341. doi:10.1007/s00253-016-7736-9

Fierer, N., Bradford, M.A., Jackson, R.B., 2007. Toward an Ecological Classification of Soil Bacteria. Ecology 88, 1354–1364. doi:10.1890/05-1839

Fierer, N., Ladau, J., Clemente, J.C., Leff, J.W., Owens, S.M., Pollard, K.S., Knight, R., Gilbert, J.A., McCulley, R.L., 2013. Reconstructing the Microbial Diversity and Function of Pre-Agricultural Tallgrass Prairie Soils in the United States. Science (80-.). 342.

Francis, C.A., Roberts, K.J., Beman, J.M., Santoro, A.E., Oakley, B.B., 2005. Ubiquity and diversity of ammonia-oxidizing archaea in water columns and sediments of the ocean. PNAS 102, 14683–8. doi:10.1073/pnas.0506625102

Frank, D.A., Groffman, P.M., 2009. Plant rhizospheric N processes: what we don't know and why we should care. Ecology 90, 1512–1519. doi:10.1890/08-0789.1

Friese, C.F., Allen, M.F., 1991. The Spread of VA Mycorrhizal Fungal Hyphae in the Soil: Inoculum Types and External Hyphal Architecture. Mycologia 83, 409. doi:10.2307/3760351

Galloway, J.N., Townsend, A.R., Erisman, J.W., Bekunda, M., Cai, Z., Freney, J.R., Martinelli, L.A., Seitzinger, S.P., Sutton, M.A., 2008. Transformation of the nitrogen cycle: recent trends, questions, and potential solutions. Science (80-.). 320, 889–92. doi:10.1126/science.1136674

Giguere, A.T., Taylor, A.E., Myrold, D.D., Bottomley, P.J., 2015. Nitrification Responses of Soil Ammonia-Oxidizing Archaea and Bacteria to Ammonium Concentrations. Soil Sci. Soc. Am. J. 79, 1366–74. doi:10.2136/sssaj2015.03.0107

Giovannetti, M., Mosse, B., 1980. An Evaluation of Techniques for Measuring Vesicular Arbuscular Mycorrhizal Infection in Roots. New Phytol. 84, 489–500. doi:10.1111/j.1469-8137.1980.tb04556.x

Glaser, K., Hackl, E., Inselsbacher, E., Strauss, J., Wanek, W., Zechmeister-Boltenstern, S., Sessitsch, A., 2010. Dynamics of ammonia-oxidizing communities in barley-planted bulk soil and rhizosphere following nitrate and ammonium fertilizer amendment. FEMS Microbiol. Ecol. 74, 575–91. doi:10.1111/j.1574-6941.2010.00970.x

Glendell, M., Brazier, R.E., 2014. Accelerated export of sediment and carbon from a landscape under intensive agriculture. Sci. Total Environ. 476–477, 643–656. doi:10.1016/j.scitotenv.2014.01.057

Gomes, N.C.M., Fagbola, O., Costa, R., Rumjanek, N.G., Buchner, A., Mendona-Hagler, L., Smalla, K., 2003. Dynamics of fungal communities in bulk and maize rhizosphere soil in the tropics. Appl. Environ. Microbiol. 69, 3758–66. doi:10.1128/AEM.69.7.3758-3766.2003

Govindarajulu, M., Pfeffer, P.E., Jin, H., Abubaker, J., Douds, D.D., Allen, J.W., Bücking, H., Lammers, P.J., Shachar-Hill, Y., 2005. Nitrogen transfer in the arbuscular mycorrhizal symbiosis. Nature 435, 819–23. doi:10.1038/nature03610

Gutknecht, J.L.M., Field, C.B., Balser, T.C., 2012. Microbial communities and their responses to simulated global change fluctuate greatly over multiple years. Glob. Chang. Biol. 18, 2256–2269. doi:10.1111/j.1365-2486.2012.02686.x

Hannula, S.E., de Boer, W., van Veen, J., Berg, G., Young, J., 2012. A 3-Year Study Reveals That Plant Growth Stage, Season and Field Site Affect Soil Fungal Communities while Cultivar and GM-Trait Have Minor Effects. PLoS One 7, e33819. doi:10.1371/journal.pone.0033819

Hargreaves, S.K., Williams, R.J., Hofmockel, K.S., 2015. Environmental Filtering of Microbial Communities in Agricultural Soil Shifts with Crop Growth. PLoS One 10, e0134345. doi:10.1371/journal.pone.0134345

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME J. 9, 1177–1194. doi:10.1038/ismej.2014.210

Haynes, R.J., 1986. Uptake and assimilation of mineral nitrogen by plants, in: Haynes, R.J. (Ed.), Mineral Nitrogen in the Plant Soil System. Academic Press, Orlando, pp. 303–378.

Herman, D.J., Johnson, K.K., Jaeger, C.H., Schwartz, E., Firestone, M.K., 2006. Root Influence on Nitrogen Mineralization and Nitrification in Rhizosphere Soil. Soil Sci. Soc. Am. J. 70, 1504–11. doi:10.2136/sssaj2005.0113

Herrmann, M., Saunders, A.M., Schramm, A., 2008. Archaea dominate the ammonia-oxidizing community in the rhizosphere of the freshwater macrophyte Littorella uniflora. Appl. Environ. Microbiol. 74, 3279–83. doi:10.1128/AEM.02802-07

Hirel, B., Tétu, T., Lea, P.J., Dubois, F., 2011. Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture. Sustainability 3, 1452–1485. doi:10.3390/su3091452

Houlden, A., Timms-Wilson, T.M., Day, M.J., Bailey, M.J., 2008. Influence of plant developmental stage on microbial community structure and activity in the rhizosphere of three field crops. FEMS Microbiol. Ecol. 65, 193–201. doi:10.1111/j.1574-6941.2008.00535.x

Hussain, Q., Liu, Y., Jin, Z., Zhang, A., Pan, G., Li, L., Crowley, D., Zhang, X., Song, X., Cui, L., 2011. Temporal dynamics of ammonia oxidizer (amoA) and denitrifier (nirK) communities in the rhizosphere of a rice ecosystem from Tai Lake region, China. Appl. Soil Ecol. 48, 210–218. doi:10.1016/j.apsoil.2011.03.004

Jaeger, C.H., Lindow, S.E., Miller, W., Clark, E., Firestone, M.K., 1999. Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Appl. Environ. Microbiol. 65, 2685–90.

Jia, Z., Conrad, R., 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. Environ. Microbiol. 11, 1658–71. doi:10.1111/j.1462-2920.2009.01891.x

Kalra, Y.P. (Ed.), 1998. Handbook of Reference Methods for Plant Analysis. Taylor & Francis Group, Boca Raton, FL. doi:10.2135/cropsci1998.0011183X003800060050x

Kamaa, M., Mburu, H., Blanchart, E., Chibole, L., Chotte, J.-L., Kibunja, C., Lesueur, D., 2011. Effects of organic and inorganic fertilization on soil bacterial and fungal microbial diversity in the Kabete long-term trial, Kenya. Biol. Fertil. Soils 47, 315–321. doi:10.1007/s00374-011-0539-3

Kandeler, E., Marschner, P., Tscherko, D., Singh Gahoonia, T., Nielsen, N.E., 2002. Microbial community composition and functional diversity in the rhizosphere of maize. Plant Soil 238, 301–312. doi:10.1023/A:1014479220689

King, A.E., Hofmockel, K.S., 2017. Diversified cropping systems support greater microbial cycling and retention of carbon and nitrogen. Agric. Ecosyst. Environ. 240, 66–76. doi:10.1016/j.agee.2017.01.040

Köhl, L., van der Heijden, M.G.A., 2016. Arbuscular mycorrhizal fungal species differ in their effect on nutrient leaching. Soil Biol. Biochem. 94. doi:10.1016/j.soilbio.2015.11.019

Kõljalg, U., Nilsson, R.H., Abarenkov, K., Tedersoo, L., Taylor, A.F.S., Bahram, M., Bates, S.T., Bruns, T.D., Bengtsson-Palme, J., Callaghan, T.M., Douglas, B., Drenkhan, T., Eberhardt, U., Dueñas, M., Grebenc, T., Griffith, G.W., Hartmann, M., Kirk, P.M., Kohout, P., Larsson, E., Lindahl, B.D., Lücking, R., Martín, M.P., Matheny, P.B., Nguyen, N.H., Niskanen, T., Oja, J., Peay, K.G., Peintner, U., Peterson, M., Põldmaa, K., Saag, L., Saar, I., Schüßler, A., Scott, J.A., Senés, C., Smith, M.E., Suija, A., Taylor, D.L., Telleria, M.T., Weiss, M., Larsson, K.-H., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. doi:10.1111/mec.12481

Kraiser, T., Gras, D.E., Gutiérrez, A.G., González, B., Gutiérrez, R.A., 2011. A holistic view of nitrogen acquisition in plants. J. Exp. Bot. 62, 1455–66. doi:10.1093/jxb/erq425

Kramer, S., Dibbern, D., Moll, J., Huenninghaus, M., Koller, R., Krueger, D., Marhan, S., Urich, T., Wubet, T., Bonkowski, M., Buscot, F., Lueders, T., Kandeler, E., 2016. Resource Partitioning between Bacteria, Fungi, and Protists in the Detritusphere of an Agricultural Soil. Front. Microbiol. 7, 1–12. doi:10.3389/fmicb.2016.01524

Kuzyakov, Y., Raskatov, A., Kaupenjohann, M., 2003. Turnover and distribution of root exudates of Zea mays. Plant Soil 254, 317–327. doi:10.1023/A:1025515708093

Li, X.G., Jia, B., Lv, J., Ma, Q., Kuzyakov, Y., Li, F., 2017. Nitrogen fertilization decreases the decomposition of soil organic matter and plant residues in planted soils. Soil Biol. Biochem. 112, 47–55. doi:10.1016/j.soilbio.2017.04.018

Licht, M., Abendroth, L.J., Elmore, R.W., Boyer, M.J., Marlay, S.K., 2011. Corn Growth and Development, PMR 1009. ed. Iowa State University Extension, Ames, Iowa.

Liebman, M., Gibson, L.R., Sundberg, D.N., Heggenstaller, A.H., Westerman, P.R., Chase, C.A., Hartzler, R.G., Menalled, F.D., Davis, A.S., Dixon, P.M., 2008. Agronomic and economic performance characteristics of conventional and low-external-input cropping systems in the central corn belt. Agron. J. 100, 600–610. doi:10.2134/agronj2007.0222

Love, M.I., Huber, W., Anders, S., 2014. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. Genome Biol. 15, 550. doi:10.1186/s13059-014-0550-8

Martinez-Garcia, M., Brazel, D.M., Swan, B.K., Arnosti, C., Chain, P.S.G., Reitenga, K.G., Xie, G., Poulton, N.J., Gomez, M.L., Masland, D.E.D., Thompson, B., Bellows, W.K., Ziervogel, K., Lo, C.-C., Ahmed, S., Gleasner, C.D., Detter, C.J., Stepanauskas, R., 2012. Capturing Single Cell Genomes of Active Polysaccharide Degraders: An Unexpected Contribution of Verrucomicrobia. PLoS One 7, e35314. doi:10.1371/journal.pone.0035314

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS One 8, e61217. doi:10.1371/journal.pone.0061217

Micallef, S.A., Channer, S., Shiaris, M.P., Colón-Carmona, A., 2009. Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. Plant Signal. Behav. 4, 777–780. doi:10.4161/psb.4.8.9229

Neumann, G., Bott, S., Ohler, M.A., Mock, H.-P., Lippmann, R., Grosch, R., Smalla, K., 2014. Root exudation and root development of lettuce (Lactuca sativa L. cv. Tizian) as affected by different soils. Front. Microbiol. 5, 1–6. doi:10.3389/fmicb.2014.00002

Ngosong, C., Gabriel, E., Ruess, L., 2012. Use of the signature Fatty Acid 16:1ω5 as a tool to determine the distribution of arbuscular mycorrhizal fungi in soil. J. Lipids 2012, 1–8. doi:10.1155/2012/236807

Nicol, G.W., Leininger, S., Schleper, C., Prosser, J.I., 2008. The influence of soil pH on the diversity, abundance and transcriptional activity of ammonia oxidizing archaea and bacteria. Environ. Microbiol. 10, 2966–2978. doi:10.1111/j.1462-2920.2008.01701.x

Nielsen, M.N., Winding, A., Binnerup, S., Munk, B., Niels, H., Hendriksen, B., Kroer, N., Hansen, B.M., Bohse Hendriksen, N., Pritchard, H., Karlson, U., 2002. Microorganisms as indicators of soil health, NERI Techn. ed. National Environmental Research Institute ©, Denmark.

Nuccio, E.E., Hodge, A., Pett-Ridge, J., Herman, D.J., Weber, P.K., Firestone, M.K., 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environ. Microbiol. 15, 1870–81. doi:10.1111/1462-2920.12081

Nunes da Rocha, U., van Elsas, J.D., van Overbeek, L.S., 2011. Verrucomicrobia subdivision 1 strains display a difference in the colonization of the leek (Allium porrum) rhizosphere. FEMS Microbiol. Ecol. 78, 297–305. doi:10.1111/j.1574-6941.2011.01156.x

Nunes da Rocha, U., Plugge, C.M., George, I., van Elsas, J.D., van Overbeek, L.S., Galantini, J., 2013. The Rhizosphere Selects for Particular Groups of Acidobacteria and Verrucomicrobia. PLoS One 8, e82443. doi:10.1371/journal.pone.0082443

Oehl, F., Sieverding, E., Ineichen, K., Mäder, P., Boller, T., Wiemken, A., 2003. Impact of Land Use Intensity on the Species Diversity of Arbuscular Mycorrhizal Fungi in Agroecosystems of Central Europe. Appl. Environ. Microbiol. 69, 2816–24. doi:10.1128/AEM.69.5.2816

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2017. vegan: Community Ecology Package. R Packag. version 2.4-3.

Otten, W., Gilligan, C.A., 1998. Effect of physical conditions on the spatial and temporal dynamics of the soil-borne fungal pathogen Rhizoctonia solani, The New Phytologist. William Wesley and Son.

Otten, W., Harris, K., Young, I.M., Ritz, K., Gilligan, C.A., 2004. Preferential spread of the pathogenic fungus Rhizoctonia solani through structured soil. Soil Biol. Biochem. 36, 203–210. doi:10.1016/J.SOILBIO.2003.09.006

Ouyang, Y., Norton, J.M., Stark, J.M., 2017. Ammonium availability and temperature control contributions of ammonia oxidizing bacteria and archaea to nitrification in an agricultural soil. Soil Biol. Biochem. 113, 161–172. doi:10.1016/j.soilbio.2017.06.010

Ouyang, Y., Norton, J.M., Stark, J.M., Reeve, J.R., Habteselassie, M.Y., 2016. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. Soil Biol. Biochem. 96, 4–15. doi:10.1016/j.soilbio.2016.01.012

Pajor, R., Falconer, R., Hapca, S., Otten, W., 2010. Modelling and quantifying the effect of heterogeneity in soil physical conditions on fungal growth. Biogeosciences 7, 3731–3740. doi:10.5194/bg-7-3731-2010

Paterson, E., Gebbing, T., Abel, C., Sim, A., Telfer, G., 2006. Rhizodeposition shapes rhizosphere microbial community structure in organic soil. New Phytol. 173, 600–610. doi:10.1111/j.1469-8137.2006.01931.x

Paulson, J.N., Stine, O.C., Bravo, H.C., Pop, M., 2013. Differential abundance analysis for microbial marker-gene surveys. Nat. Methods 10, 1200–2. doi:10.1038/nmeth.2658

Pepe-Ranney, C., Campbell, A.N., Koechli, C.N., Berthrong, S., Buckley, D.H., 2016. Unearthing the Ecology of Soil Microorganisms Using a High Resolution DNA-SIP Approach to Explore Cellulose and Xylose Metabolism in Soil. Front. Microbiol. 7. doi:10.3389/fmicb.2016.00703

Pimentel, D., Burgess, M., 2013. Soil Erosion Threatens Food Production. Agriculture 3, 443–463. doi:10.3390/agriculture3030443

Prosser, J.I., Nicol, G.W., 2008. Relative contributions of archaea and bacteria to aerobic ammonia oxidation in the environment. Environ. Microbiol. 10, 2931–2941. doi:10.1111/j.1462-2920.2008.01775.x

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., Glockner, F.O., 2013. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 41, D590–D596. doi:10.1093/nar/gks1219

Raun, W.R., Johnson, G. V., 1999. Improving Nitrogen Use Efficiency for Cereal Production. Agron. J. 91, 357. doi:10.2134/agronj1999.00021962009100030001x

Raynaud, X., 2010. Soil properties are key determinants for the development of exudate gradients in a rhizosphere simulation model. Soil Biol. Biochem. 42, 210–219. doi:10.1016/J.SOILBIO.2009.10.019

Richardson, A.E., Barea, J.-M., McNeill, A.M., Prigent-Combaret, C., 2009. Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. Plant Soil 321, 305–339. doi:10.1007/S11104-009-9895-2

Roberts, D.W., 2016. labdsv: Ordination and Multivariate Analysis for Ecology [WWW Document]. URL https://cran.r-project.org/package=labdsv (accessed 9.27.17).

Rotthauwe, J.H., Witzel, K.P., Liesack, W., 1997. The ammonia monooxygenase structural gene amoA as a functional marker: molecular fine-scale analysis of natural ammonia-oxidizing populations. Appl. Environ. Microbiol. 63, 4704–4712.

Rudisill, M.A., Turco, R.F., Hoagland, L.A., 2016. Fertility practices and rhizosphere effects alter ammonia oxidizer community structure and potential nitrification activity in pepper production soils. Appl. Soil Ecol. 99, 70–77. doi:10.1016/j.apsoil.2015.10.011

Sharma, B., Ahlert, R.C., 1977. Nitrification and nitrogen removal. Water Res. 11, 897–925. doi:10.1016/0043-1354(77)90078-1

Schoch, C.L., Sung, G.-H., López-Giráldez, F., Townsend, J.P., Miadlikowska, J., Hofstetter, V., Robbertse, B., Matheny, P.B., Kauff, F., Wang, Z., Gueidan, C., Andrie, R.M., Trippe, K., Ciufetti, L.M., Wynns, A., Fraker, E., Hodkinson, B.P., Bonito, G., Groenewald, J.Z., Arzanlou, M., Sybren de Hoog, G., Crous, P.W., Hewitt, D., Pfister, D.H., Peterson, K., Gryzenhout, M., Wingfield, M.J., Aptroot, A., Suh, S.-O., Blackwell, M., Hillis, D.M., Griffith, G.W., Castlebury, L.A., Rossman, A.Y., Lumbsch, H.T., Lücking, R., Büdel, B., Rauhut, A., Diederich, P., Ertz, D., Geiser, D.M., Hosaka, K., Inderbitzin, P., Kohlmeyer, J., Volkmann-Kohlmeyer, B., Mostert, L., O'Donnell, K., Sipman, H., Rogers, J.D., Shoemaker, R.A., Sugiyama, J., Summerbell, R.C., Untereiner, W., Johnston, P.R., Stenroos, S., Zuccaro, A., Dyer, P.S., Crittenden, P.D., Cole, M.S., Hansen, K., Trappe, J.M., Yahr, R., Lutzoni, F., Spatafora, J.W., 2009. The Ascomycota Tree of Life: A Phylum-wide Phylogeny Clarifies the Origin and Evolution of Fundamental Reproductive and Ecological Traits. Syst. Biol. 58, 224–239. doi:10.1093/sysbio/syp020

Smil, V., 1999. Nitrogen in crop production: An account of global flows. Global Biogeochem. Cycles 13, 647–662. doi:10.1029/1999GB900015

Smith, D.P., Peay, K.G., 2014. Sequence Depth, Not PCR Replication, Improves Ecological Inference from Next Generation DNA Sequencing. PLoS One 9, e90234. doi:10.1371/journal.pone.0090234

Sterngren, A.E., Hallin, S., Bengtson, P., 2015. Archaeal Ammonia Oxidizers Dominate in Numbers, but Bacteria Drive Gross Nitrification in N-amended Grassland Soil. Front. Microbiol. 6, 1–8. doi:10.3389/fmicb.2015.01350

Sugiyama, A., Vivanco, J.M., Jayanty, S.S., Manter, D.K., 2010. Pyrosequencing Assessment of Soil Microbial Communities in Organic and Conventional Potato Farms. Plant Dis. 94, 1329–1335.

Tanaka, Y., Matsuzawa, H., Tamaki, H., Tagawa, M., Toyama, T., Kamagata, Y., Mori, K., 2017. Isolation of Novel Bacteria Including Rarely Cultivated Phyla, Acidobacteria and Verrucomicrobia, from the Roots of Emergent Plants by Simple Culturing Method. Microbes Environ. ME17027. doi:10.1264/jsme2.ME17027

Tanaka, Y., Yano, K., 2005. Nitrogen delivery to maize via mycorrhizal hyphae depends on the form of N supplied. Plant, Cell Environ. 28, 1247–1254. doi:10.1111/j.1365-3040.2005.01360.x

Taylor, A.R., Bloom, A.J., 1998. Ammonium, nitrate, and proton fluxes along the maize root. Plant, Cell Environ. 21, 1255–1263. doi:10.1046/j.1365-3040.1998.00357.x

Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. Proc. Natl. Acad. Sci. U. S. A. 108, 20260–4. doi:10.1073/pnas.1116437108

Tomer, M.D., Liebman, M., 2014. Nutrients in soil water under three rotational cropping systems, Iowa, USA. Agric. Ecosyst. Environ. 186, 105–114. doi:10.1016/j.agee.2014.01.025

Tourna, M., Freitag, T.E., Nicol, G.W., Prosser, J.I., 2008. Growth, activity and temperature responses of ammonia-oxidizing archaea and bacteria in soil microcosms. Environ. Microbiol. 10, 1357–64. doi:10.1111/j.1462-2920.2007.01563.x

Van Loo, M., Dusar, B., Verstraeten, G., Renssen, H., Notebaert, B., D'Haen, K., Bakker, J., 2017. Human induced soil erosion and the implications on crop yield in a small mountainous Mediterranean catchment (SW-Turkey). Catena 149, 491–504. doi:10.1016/j.catena.2016.08.023

Veresoglou, S.D., Chen, B., Rillig, M.C., 2012. Arbuscular mycorrhiza and soil nitrogen cycling. Soil Biol. Biochem. 46, 53–62. doi:10.1016/j.soilbio.2011.11.018

Veresoglou, S.D., Sen, R., Mamolos, A.P., Veresoglou, D.S., 2011. Plant species identity and arbuscular mycorrhizal status modulate potential nitrification rates in nitrogen-limited grassland soils. J. Ecol. 99, 1339–1349. doi:10.1111/j.1365-2745.2011.01863.x

Verhagen, F.J., Laanbroek, H.J., 1991. Competition for Ammonium between Nitrifying and Heterotrophic Bacteria in Dual Energy-Limited Chemostats. Appl. Environ. Microbiol. 57, 3255–63.

Verhagen, F.J.M., Laanbroek, H.J., Woldendrop, J.W., 1995. Competition for ammonium between plant roots and nitrifying and heterotrophic bacteria and the effects of protozoan grazing. Plant Soil 170, 241–250. doi:10.1007/BF00010477

Vitousek, P.M., Aber, J.D., Howarth, R.W., Likens, G.E., Matson, P.A., Schindler, D.W., Schlesinger, W.H., Tilman, D.G., 1997. Human Alteration of the Global Nitrogen Cycle: Sources and Consequences. Ecol. Appl. 7, 737–750. doi:10.1890/1051-0761(1997)007[0737:HAOTGN]2.0.CO;2

Ward, N.L., Challacombe, J.F., Janssen, P.H., Henrissat, B., Coutinho, P.M., Wu, M., Xie, G., Haft, D.H., Sait, M., Badger, J., Barabote, R.D., Bradley, B., Brettin, T.S., Brinkac, L.M., Bruce, D., Creasy, T., Daugherty, S.C., Davidsen, T.M., DeBoy, R.T., Detter, J.C., Dodson, R.J., Durkin, A.S., Ganapathy, A., Gwinn-Giglio, M., Han, C.S., Khouri, H., Kiss, H., Kothari, S.P., Madupu, R., Nelson, K.E., Nelson, W.C., Paulsen, I., Penn, K., Ren, Q., Rosovitz, M.J., Selengut, J.D., Shrivastava, S., Sullivan, S.A., Tapia, R., Thompson, L.S., Watkins, K.L., Yang,

Q., Yu, C., Zafar, N., Zhou, L., Kuske, C.R., 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. Appl. Environ. Microbiol. 75, 2046–56. doi:10.1128/AEM.02294-08

Watt, M., Silk, W.K., Passioura, J.B., 2006. Rates of Root and Organism Growth, Soil Conditions, and Temporal and Spatial Development of the Rhizosphere. Ann. Bot. 97, 839–855. doi:10.1093/aob/mcl028

Wessén, E., Hallin, S., 2011. Abundance of archaeal and bacterial ammonia oxidizers – Possible bioindicator for soil monitoring, Ecological Indicators. doi:10.1016/j.ecolind.2011.04.018

Wessén, E., Nyberg, K., Jansson, J.K., Hallin, S., 2010. Responses of bacterial and archaeal ammonia oxidizers to soil organic and fertilizer amendments under long-term management. Appl. Soil Ecol. 45, 193–200. doi:10.1016/j.apsoil.2010.04.003

Wieland, G., Neumann, R., Backhaus, H., 2001. Variation of Microbial Communities in Soil, Rhizosphere, and Rhizoplane in Response to Crop Species, Soil Type, and Crop Development. Appl. Environ. Microbiol. 67, 5849–5854. doi:10.1128/AEM.67.12.5849-5854.2001

Wu, Y., Lu, L., Wang, B., Lin, X., Zhu, J., Cai, Z., Yan, X., Jia, Z., 2011. Long-Term Field Fertilization Significantly Alters Community Structure of Ammonia-Oxidizing Bacteria rather than Archaea in a Paddy Soil. Soil Sci. Soc. Am. J. 75, 1431. doi:10.2136/sssaj2010.0434

Xu, Y., Wang, G., Jin, J., Liw, J., Xhang, Q., Liu, X., 2009. Bacterial communities in soybean rhizosphere in response to soil type, soybean genotype, and their growth stage. Soil Biol. Biochem. 41, 919–925. doi:10.1016/J.SOILBIO.2008.10.027

Yin, C., Mueth, N., Hulbert, S., Schlatter, D., Paulitz, T.C., Schroeder, K., Prescott, A., Dhingra, A., 2017. Bacterial Communities on Wheat Grown Under Long-Term Conventional Tillage and No-Till in the Pacific Northwest of the United States. Phytobiomes 1, 83–90. doi:10.1094/PBIOMES-09-16-0008-R

Ying, J., Li, X., Wang, N., Lan, Z., He, J., Bai, Y., 2017. Contrasting effects of nitrogen forms and soil pH on ammonia oxidizing microorganisms and their responses to long-term nitrogen fertilization in a typical steppe ecosystem. Soil Biol. Biochem. 107, 10–18. doi:10.1016/j.soilbio.2016.12.023

Yurgel, S.N., Douglas, G.M., Comeau, A.M., Mammoliti, M., Dusault, A., Percival, D., Langille, M.G.I., 2017. Variation in Bacterial and Eukaryotic Communities Associated with Natural and Managed Wild Blueberry Habitats. Phytobiomes 1, 102–113. doi:10.1094/PBIOMES-03-17-0012-R

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C., Chu, H., 2016. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. Soil Biol. Biochem. 92, 41–49. doi:10.1016/J.SOILBIO.2015.09.018

Zhang, Y., Crous, P.W., Schoch, C.L., Hyde, K.D., 2012. Pleosporales. Fungal Divers. 53, 1–221. doi:10.1007/s13225-011-0117-x

Zhang, B., He, H., Ding, X., Zhang, X., Zhang, X., Yang, X., Filley, T.R., 2012. Soil microbial community dynamics over a maize (Zea mays L.) growing season under conventional- and no-tillage practices in a rainfed agroecosystem. Soil Tillage Res. 124, 153–160. doi:10.1016/j.still.2012.05.011

Zhang, Y., Schoch, C.L., Fournier, J., Crous, P.W., de Gruyter, J., Woudenberg, J.H.C., Hirayama, K., Tanaka, K., Pointing, S.B., Spatafora, J.W., Hyde, K.D., 2009. Multi-locus phylogeny of Pleosporales: a taxonomic, ecological and evolutionary re-evaluation. Stud. Mycol. 64, 85–102S5. doi:10.3114/sim.2009.64.04

Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q., Shen, W., 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. Plant Soil 326, 511–522. doi:10.1007/s11104-009-9988-y

Zhu, S., Vivanco, J.M., Manter, D.K., 2016. Nitrogen fertilizer rate affects root exudation, the rhizosphere microbiome and nitrogen-use-efficiency of maize. Appl. Soil Ecol. 107, 324–333. doi:10.1016/j.apsoil.2016.07.009

APPENDIX A: AMMONIA OXIDIER POPULATION SIZES AND CUMULATIVE AMMONIUM AND NITRATE MEASUREMENTS

Background

Ammonia oxidizers are an ecologically important group of bacteria and archaea (AOB and AOA) which perform the rate limiting step of nitrification, the conversion of ammonium to nitrate. Nitrification is a major source of nitrate leaching in agricultural systems, so we are interested in understanding the environmental and biotic factors that control the population size and activities of AOB and AOB in soils. Studies have shown that plant roots can alter the population sizes and activities of AOA and AOB (Ai et al., 2013; Dias et al., 2012; Hussain et al., 2011; Herman et al. 2006), but few studies have investigated how the rhizosphere may affect ammonia oxidizers differently based on agricultural management. Here, we investigated changes in AOB and AOA abundances in the bulk and rhizosphere soils of two contrasting agricultural systems over the course of maize development.

Methods

Bulk soil and rhizosphere soil were collected at four points in maize development as described in Chapter 2 from the Marsden field site. SYBR Green qPCR was conducted to assess AOB and AOA community sizes as described in Chapter 3. PRS Probes (Western Ag Innovations, Saskatoon CA) were buried one week prior to sampling between rows and one inch from the corn stalk (bulk and rhizosphere respectively) to assess ammonium and nitrate accumulation. Probes were collected at sampling, rinsed, and sent to Western Ag Innovations for ion extraction and measurement

References

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W., 2013. Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. Soil Biology and Biochemistry 57, 30–42. doi:10.1016/j.soilbio.2012.08.003

Dias, A.C.F., Hoogwout, E.F., Pereira e Silva, M. de C., Salles, J.F., van Overbeek, L.S., van Elsas, J.D., 2012. Potato cultivar type affects the structure of ammonia oxidizer communities in field soil under potato beyond the rhizosphere. Soil Biology and Biochemistry 50, 85–95. doi:10.1016/j.soilbio.2012.03.006

Hussain, Q., Liu, Y., Jin, Z., Zhang, A., Pan, G., Li, L., Crowley, D., Zhang, X., Song, X., Cui, L., 2011. Temporal dynamics of ammonia oxidizer (amoA) and denitrifier (nirK) communities in the rhizosphere of a rice ecosystem from Tai Lake region, China. Applied Soil Ecology 48, 210–218. doi:10.1016/j.apsoil.2011.03.004

Herman, D.J., Johnson, K.K., Jaeger, C.H., Schwartz, E., Firestone, M.K., 2006. Root Influence on Nitrogen Mineralization and Nitrification in Rhizosphere Soil. Soil Science Society of America Journal 70, 1504–11. doi:10.2136/sssaj2005.0113

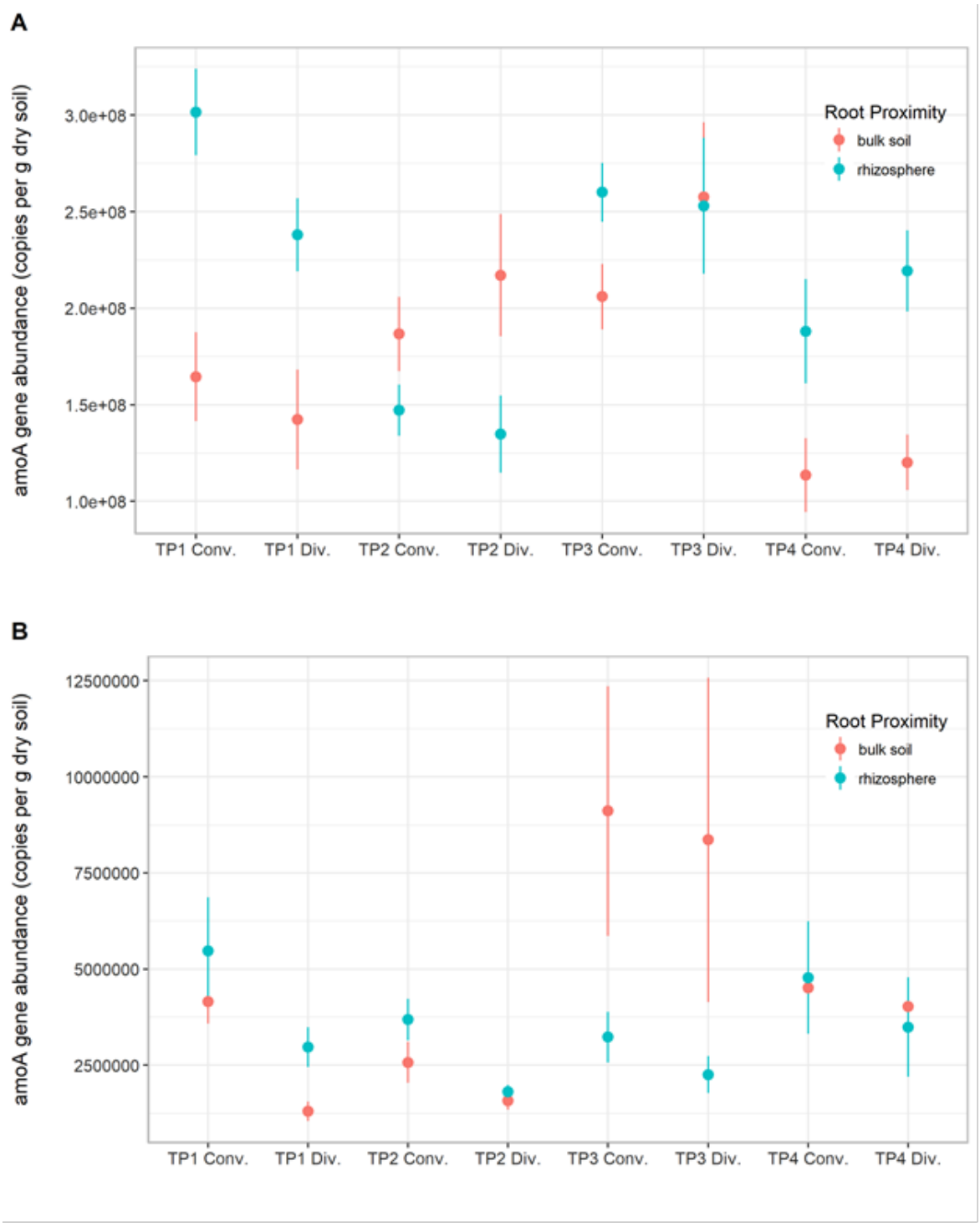

Figure A1. Ammonia oxidizer abundance in field bulk and rhizosphere soil as quantified by qPCR. **A)** Ammonia-oxidizing archaea gene abundance and **B)** ammonia-oxidizing bacteria gene abundance.

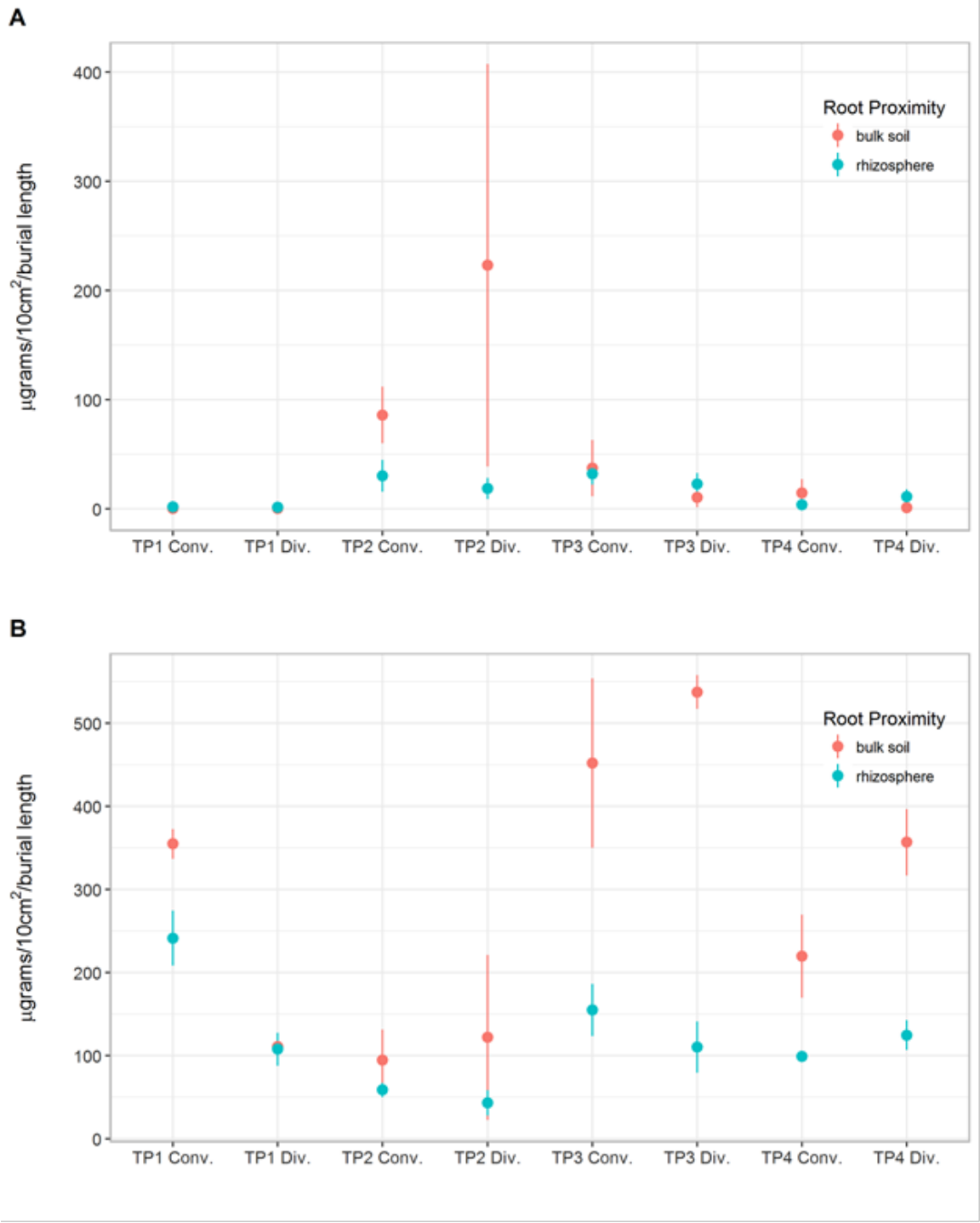

Figure A2. Accumulation of ammonium and nitrate over a one-week burial period as measured by PRS cation and anion probes. **A)** Ammonium accumulation and **B)** nitrate accumulation.

APPENDIX B: EFFECTS OF ARBUSCULAR MYCCORHIZAE ON PROKARYOTIC AND FUNGAL COMMUNITY COMPOSITION

Background

Recent studies have found that AMF can alter the prokaryotic community structure of soils (Lioussane et al., 2010; Marschner and Baumann, 2003; Nuccio et al., 2013; Rillig et al., 2006). However, no studies have investigated how AMF in agricultural soils alter surrounding microbial communities. Furthermore, effects on the overall fungal communities have been overlooked in these studies. Here, we sequenced the rhizosphere and bulk soil prokaryotic and fungal communities of AMF deficient (knock-out of the dmi1 gene) and AMF proficient (wild type progenitor) maize genotypes to investigate the impact of AMF on soil communities in differing agroecosystems.

Methods

AMF deficient (AMF-) and AMF proficient (AMF+) maize seeds were surface sterilized, pre-germinated, and planted into rhizotrons (Jaeger et al., 1999) packed with conventional and diversified soil collected from the Marsden field site on 5/6/16. Plants were grown and soils were harvested as described in Chapter 3. DNA was extracted, sequencing conducted, and data quality filtering and preprocessing was conducted as described in Chapter 2. Principle Coordinate Analysis (PCoA) ordinations were constructed to visualize differences in β diversity and permutational analysis of variance (PERMANOVA) with 9999 permutations was used to assess significant differences in β diversity between treatments using the ‘vegan’ and ‘phyloseq’ R packages in R (version 3.3) (Oksanen et al., 2017; McMurdie and Holmes, 2013).

References

Jaeger, C.H., Lindow, S.E., Miller, W., Clark, E., Firestone, M.K., 1999. Mapping of sugar and amino acid availability in soil around roots with bacterial sensors of sucrose and tryptophan. Applied and Environmental Microbiology 65, 2685–90.

Lioussanne, L., Perreault, F., Jolicoeur, M., St-Arnaud, M., 2010. The bacterial community of tomato rhizosphere is modified by inoculation with arbuscular mycorrhizal fungi but unaffected by soil enrichment with mycorrhizal root exudates or inoculation with Phytophthora nicotianae. Soil Biology and Biochemistry 42, 473–483. doi:10.1016/j.soilbio.2009.11.034

Marschner, P., Baumann, K., 2003. Changes in bacterial community structure induced by mycorrhizal colonisation in split-root maize. Plant and Soil 251, 279–289. doi:10.1023/A:1023034825871

McMurdie, P.J., Holmes, S., 2013. phyloseq: An R Package for Reproducible Interactive Analysis and Graphics of Microbiome Census Data. PLoS ONE 8, e61217. doi:10.1371/journal.pone.0061217

Nuccio, E.E., Hodge, A., Pett-Ridge, J., Herman, D.J., Weber, P.K., Firestone, M.K., 2013. An arbuscular mycorrhizal fungus significantly modifies the soil bacterial community and nitrogen cycling during litter decomposition. Environmental Microbiology 15, 1870–81. doi:10.1111/1462-2920.12081

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., Mcglinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Henry, M., Stevens, H., Szoecs, E., Wagner, H., 2017. vegan: Community Ecology Package. R Package Version 2.4-3.

Rillig, M.C., Mummey, D.L., Ramsey, P.W., Klironomos, J.N., Gannon, J.E., 2006. Phylogeny of arbuscular mycorrhizal fungi predicts community composition of symbiosis-associated bacteria. FEMS Microbiology Ecology 57, 389–395. doi:10.1111/j.1574-6941.2006.00129.x

Table B1. Permutational analysis of variance of β diversity. Results are based on 9999 permutations.

	Prokaryotic		Fungal	
Treatment Effect	**R^2**	**P-value**	**R^2**	**P-value**
Cropping system	0.055	0.0010	0.052	0.0002
Root proximity	0.622	0.0001	0.615	0.0001
Genotype	0.031	0.0001	0.024	0.0032
Cropping system * Root proximity	0.033	0.0040	0.046	0.0003
Cropping system * Genotype	0.006	0.2270	0.005	0.2742
Genotype * Root proximity	0.041	0.0009	0.032	0.0059
Cropping System * Root proximity * Genotype	0.008	0.4420	0.005	0.8114

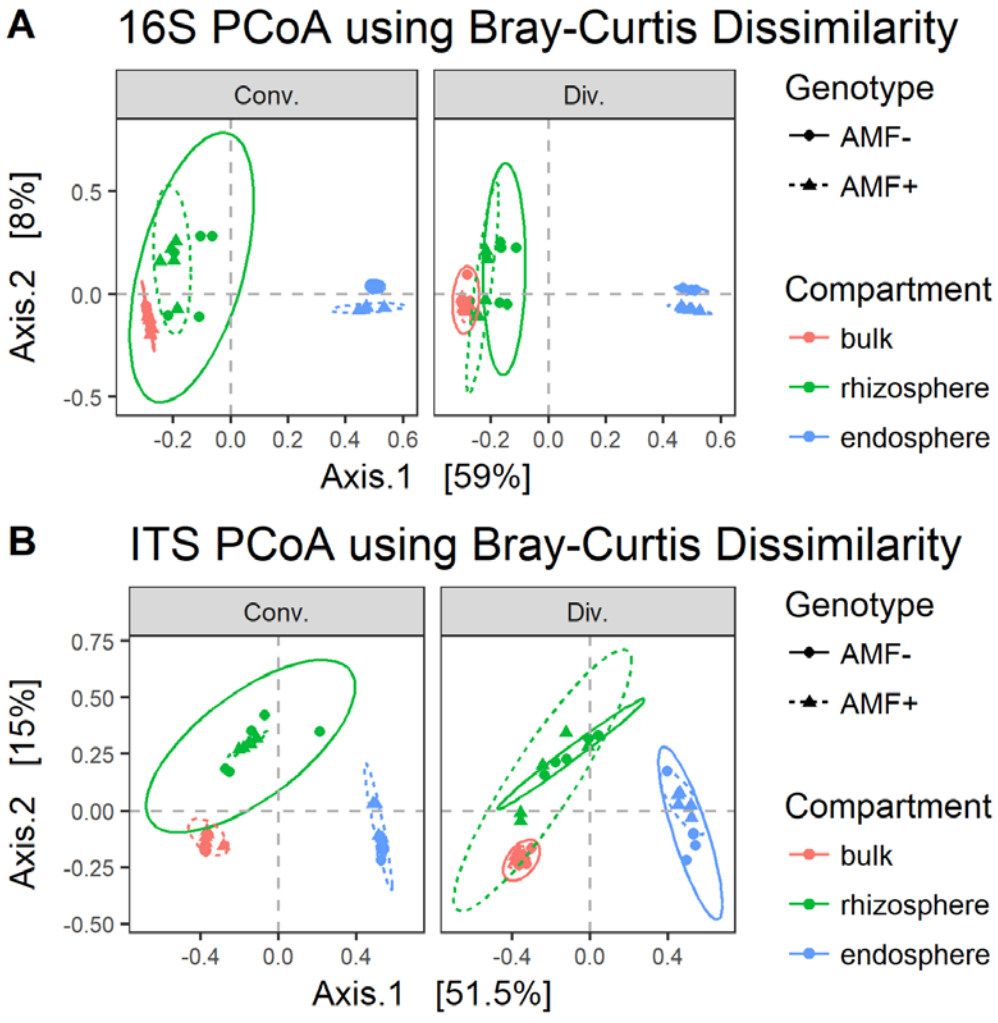

Figure B1. Principle coordinates analysis ordinations of Bray-Curtis dissimilarity. AMF- refers to the AMF deficient maize genotype while AMF+ refers to AMF proficient maize genotype.

Table B2. Additional Soil Properties Before and After Experiment. These values show additional measurements that were taken for the experiment described in chapter 2 that were not discussed in the main body. Potassium and iron measurements were taken via Mehlich-3 analysis and Total Carbon (C) was measured via combustion. GWC means gravimetric water content and was measured by oven drying the soil after the experiment. Numbers represent means and standard error (n=5).

	Treatment					
	Mycorrhization	**Soil Management**	**Potassium (mg/kg)**	**Iron (mg/kg)**	**Total C (%)**	**GWC**
Before	N/A	Conventional	202.3 ± 7.9	102 ± 8.1	2.5 ± 0.04	N/A
	N/A	Diversified	136.7 ± 10.7	123.3 ± 2.8	2.4 ± 0.07	N/A
After	AMF-	Conventional	222.6 ± 5.4	119.4 ± 6.5	2.4 ± 0.02	0.19 ± 0.01
	AMF-	Diversified	164.8 ± 4.8	156.2 ± 3.3	2.3 ± 0.06	0.17 ± 0.01
	AMF+	Conventional	179.4 ± 3.3	129 ± 3.6	2.4 ± 0.05	0.15 ± 0.01
	AMF+	Diversified	143.8 ± 5.3	152 ± 2.7	2.4 ± 0.04	0.16 ± 0.01

Table B3. Statistics of Additional Soil Properties. These numbers represent P-values as determined by two-way ANOVA.

Main Effect	**Potassium**	**Iron**	**Total C**	**GWC**
Before				
Soil Management	0.008	0.105	0.924	N/A
After				
Soil Management	< 0.001	< 0.001	0.259	0.939
Mycorrhization	< 0.001	0.535	0.622	0.018
Interaction	0.033	0.125	0.742	0.134